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CARBON AND NITROGEN ASSIMILATION, DINITROGEN FIXATION IN FABA BEAN (*VICIA FABA* L.), AND MICROBIAL BIOMASS IN SOIL-PLANT SYSTEMS (FABA BEAN, CANOLA, BARLEY AND SUMMER FALLOW) ON A GRAY LUVISOL.

BY

JI-DONG GU

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MASTER OF SCIENCE

IN

SOIL MICROBIOLOGY & BIOCHEMISTRY

DEPARTMENT OF SOIL SCIENCE

EDMONTON, ALBERTA

FALL 1988

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled CARBON AND NITROGEN ASSIMILATION, DINITROGEN FIXATION IN FABA BEAN (*VICIA FABA* L.), AND MICROBIAL BIOMASS IN SOIL-PLANT SYSTEMS (FABA BEAN, CANOLA, BARLEY AND SUMMER FALLOW) ON A GRAY LUVISOL submitted by JI-DONG GU in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in SOIL MICROBIOLOGY & BIOCHEMISTRY.

.....
Supervisor

.....

.....

.....

Date: July 29, 1988

To my mother, Ming-Jie Liu for her love.

ABSTRACT

Reference crops for measuring dinitrogen fixation by faba bean were evaluated at the Soil Science Plots at Breton in 1987. Crop growth rate of tops and roots were examinaed by conducting four samplings. Symbiotic dinitrogen fixation and soil microbial biomass C and N were studied as well. Crop roots were extracted from soil by a root-washing technique for three depths (0-10, 10-20 and 20-27cm). Above ground crop materials were partitioned into various components. Both canola and barley were evaluated as reference crops. Applied N-15 enriched urea-N was measured in various components of crop tops, crop roots, crop residues from the previous crop in soil, crop residues on the soil surface, flush N from microbial biomass, and inorganic NH_4^+ and NO_3^-. N-15 excess was highest in crop tops, crop roots and residues on the soil surface.

Dry matter and N assimilated in faba bean differed from those in canola and barley when crops reached regenerative growth. There was no significant difference in dry matter and N assimilated between the legume (faba bean) and nonlegumes (canola and barley) when the crops were still in vegetative growth. Throughout the growing season, there was no significant difference in dry matter and N accumulated in crop tops between canola and barley. Both canola and barley were equally effective as reference crops for faba bean in the N-15 dilution method to quantify dinitrogen fixation. Variation of N-15 excess in different components of crops was found, but was not great.

Symbiotically fixed dinitrogen was estimated to be 183-199 kg N ha^{-1} yr^{-1} in faba bean tops with canola and barley as reference crop. Between 18-22 kg N ha^{-1} yr^{-1} in roots layer in the 0-27cm roots was found to be originally from the atmosphere with the N-15 dilution method. The peak rate of dinitrogen fixation was calculated to be 4.0-4.7 kg N ha^{-1} day^{-1}. The total N difference method was within 10% of the N-15 dilution technique's quantifying dinitrogen fixation in faba beans on this Gray Luvisol.

Soil microbial biomass C was significantly affected by cropping; and was higher in faba bean than in canola, barley or summer fallow by 44, 39 and 167% on average over four samplings. Among three ways to calculate flush N, NH_4^+ in fumigated soil without a control gives results which deviated from those considering a control by 1-2, 1-6, 1 and 1% for soil cropped to faba bean, barley, canola and summer fallow, respectively.

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CHAPTER 1. INTRODUCTION

N status is of great concern in present agriculture because most arable soils are deficient in N available to crops (Stevenson 1982). Gray Luvisols, which occupy 40% of the land cultivated in Alberta (Bentley *et al.* 1971), have long been studied with regard to their improvement for use in crop production (Newton 1954) and are characteristically low in available nutrients, have poor soil structure, and are moderately acidic. Symbiotically fixed N can be readily available to subsequent crops (Ladd *et al.* 1986) and can significantly reduce input costs and conserve N in organic form in sustainable farming systems. New research which involves rotations with faba beans on the Gray Luvisol at the Breton plots has been directed toward self sustaining agro-ecosystems to achieve the goals of improving soil physical and chemical properties and to understand different cropping systems better (Robertson and McGill 1983). Biological nitrogen fixation is regulated by changes in concentrations of inorganic N present. Therefore, a goal of this research project was to quantify dinitrogen fixation by faba bean (*Vicia. faba* L.) on this low fertility soil at the Breton plots using the N-15 dilution technique.

Faba bean is an important legume in world agriculture, especially in Asia and Africa. China has the largest cultivated area and longest history of production for human consumption (Hawtin and Hebblethwaite 1983). It has drawn wide attention recently in Western Canada due to its agronomic performance and high protein content (Evans *et al.* 1972). Not only is the high protein in seeds a good source of nutritional needs for humans, but also the straw is used to feed animals (Patriquin *et al.* 1981). In addition, Zapata *et al.* (1987) reported 209 kg N ha^{-1} can be fixed in faba bean tops from the atmosphere, which corresponded to 79% of the N in faba bean, suggesting this crop is capable of very high fixation rates. As the price of fuel continues to increase, sustainable agriculture has been highly recommended for low input and steady output (Patriquin 1986; Sprent 1986). It is also hoped that rotational practice could be used to synchronize conservation and release of nutrients to crops (McGill and Myers 1987).

Precise quantification of dinitrogen fixation is essential in evaluating its effects in agriculture. Several methods have been used in dinitrogen fixation studies, including: total different method (Bergersen 1980; Lepo and Ferrenbach 1987), acetylene reduction (Turner and Gibson 1980; Upchurch 1987), N-15 isotope techniques (Bergersen 1980; Focht and Poth 1987) and ureide assay (Atkins 1982; Glenister and LaRue 1987). The biochemical approach for quantifying dinitrogen fixation in *V. faba* is not possible

because ureides are not produced in this legume (Schubert 1986). Most methods suffer serious limitations, and therefore may not yield accurate quantitative estimates of the amount of dinitrogen fixed by a legume. The acetylene reduction assay has been widely used, profoundly affecting the progress of research on dinitrogen fixation (Witty and Minchin 1988) but does not provide integrated fixation data. McAuliffe *et al.* (1958) significantly advanced dinitrogen fixation studies by proposing N-15 isotope techniques; which can be classified into either the isotope dilution or A-value method.

Currently, the isotope dilution method seems to be more popular than the A-value approach (Chalk 1985; Danso 1988; Hauck and Bystron 1970). Even though the N-15 dilution method is the only one that differentiates between soil and atmospheric nitrogen, the requirement of a reference crop for the legume has created difficulties in precise quantification and caused ambiguities when comparing results (Phillips *et al.* 1986; Weaver 1986). The precision and accuracy of dinitrogen fixation estimates made with N-15 techniques are strongly influenced by the reference crop used to assess the $^{15}N/^{14}N$ ratio of the available nitrogen in the soil. Therefore, there is a serious need to evaluate the validity of various reference crops. Meeting that need forms one component of this research project. Since direct verification of a reference crop is difficult, two reference crops, canola (*Brassica napus* L.) and barley (*Hordeum vulgare* L.) were used to determine if they were equally applicable and were valid reference crops for faba bean (*Vicia faba* L.) in dinitrogen fixation studies, and if calculated quantities of dinitrogen fixation varied when using either of them as a reference crop.

Dynamics of soil N reflect mineralization and immobilization processes which are in turn controlled by microorganisms (Jansson 1958; Paul and Juma 1981). Microflora and microfauna play a central role in carbon and energy flow of the terrestrial ecosystem (Elliott *et al.* 1984). Quantity of microbial biomass and transfer of N through this fraction of soil organic matter as revealed by N-15 tracer studies, is crucial for understanding soil-plant systems. Influences of cultivation on microbial biomass affects the amount of applied N-15 found in microbial biomass, and may consequently cause errors in quantifying dinitrogen fixation in a legume if microbial communities differ fundamentaly between a legume and nonlegumes. Although microbial biomass is currently determined by the chloroform fumigation and incubation technique (CFIT) (Jenkinson and Powlson 1976a; 1976b), results often disagree and caution has been urged in assigning the proportion of the biologically active organic matter as microbial biomass (McGill *et al.* 1986; Paul and Voroney 1984).

This study was conducted at the Soil Science Plots at Breton, which have been in operation since 1929 and are located 110 km southwest of Edmonton. Our aims in this study were : (1) to compare crop top development and crop root distribution by depth; (2) to quantify dinitrogen fixation in legume, faba bean; and (3) to estimate changes in microbial biomass C and N, and N-15 incorporated into microbial biomass under different crops and bare fallow. During the summer of 1987, experiments were conducted to obtain detailed data on above ground components of each of the three crops (barley, canola and faba beans), below ground roots by depth, past crop residue remains distributed throughout the soil, and microbial biomass C and N. These data will assist later simulation modelling of C and N allocation and translocation, in these three agronomically important crops. It may help to understand interactions between roots and soil to further improve soil properties and aid management of this soil by crop rotation.

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CHAPTER 2. DRY MATTER PRODUCTION, AND ACCUMULATION OF C AND N AMONG ABOVE AND BELOW GROUND COMPONENTS OF FABA BEAN, CANOLA, AND BARLEY ON A GRAY LUVISOL[1]

2.1 Introduction

The quantities of N found in different crops and in various parts of a crop vary greatly with species and environments in which the crops are grown. Crops are a major sink for inorganic N from the soil, and the N removed by harvesting is a major outflow from the system. Precise differentiation of N into plant parts has been determined for corn (Hanway 1962), soybeans (Hanway and Weber 1971) and for small grains (Boatwright and Haas 1961). Dry matter production and N uptake by wheat reached a maximum at heading, soft dough, and maturity on NP, N, and unfertilized plots, respectively (Boatwright and Haas 1961). Differences in soil fertility influenced the amounts of N, P and K taken up by corn plants, but did not markedly change the seasonal pattern of uptake and distribution of these elements in the plants (Brouwer 1962a; 1962b; Hanway 1962). Attention to distribution and morphological characteristics of crop root sytems is increasing (Diggle 1988; Hansson and Andren 1986; 1987; Hansson and Steen 1984; Kono *et al.* 1987a; 1987b; Pettersson *et al* 1986; Sattelmacher 1987; Yamauchi *et al.* 1987a; 1987b). However, few data have been reported on the amounts of N contained in roots due to the difficulty of obtaining representative samples and poor availability of techniques to separate roots from the soil matrix and from dead organic debris (Hansson *et al.* 1987). Roots of unfertilized barley accounted for a higher percentage of total dry matter produced and also a higher amount of accumulated N than in fertilized barley (Hansson *et al.* 1987).

Maintaining a steady state of soil organic matter (SOM) has become one goal for sustaining agricutural production systems. Residue return and growth of legumes in rotation are two approaches to a solution. The value of plant residues to soil fertility will depend upon the amount applied, N content, relative availability of nutrients and so on. Information on the N contents of plant components is important to decisions concerning residue decomposition and management practices, such as N fertilizer application. Control of soil N dynamics was examined from an architectural aspect by McGill and Myers (1987). They suggested altering system architecture to conserve nutrient resources without reliance on chemical inhibitors etc. Monreal *et al.* (1987) provided a practical example of

[1]A version of this chapter will be submitted for publication by Gu, J.-D. and W.B. McGill.

this to control N fertilizer transformations. Fyles and McGill (1987) concluded that differences in internal structure and surface area of several forest litters examined influenced the rates at which they decomposed. There are differences between species of grasses regarding the amount of N that is available through decomposition for the next crop. The N in roots of several species of grass ranged from 56 to 150 kg ha^{-1} within 60cm (Haas 1958). Although leaves and litter of legumes *Desmodium intortum* cv. Greenleaf and *Phaseolus astropureus* cv. Siratro had similar N and lignin contents, more N was mineralized from the former species (Vallis and Jones 1973). They suggested that the much higher polyphenol content in the leaves of *D. intortum* was partly responsible for this difference in mineralization. Although there is considerable N present in grassland soils, only small quantities of mineral N exist unless plough-up of sod is practised.

N translocation can be realistically presented in simulation models of the N cycle (Clark 1977; McGill *et al.* 1981; Reuss and Innis 1977). There remains the major problem of assigning flow ratio from diverse plant residues and soil organic matter to the available N pool. The net annual primary production and its N content are measurable. Plant material is a chemically complex substrate containing both easily and difficultly decomposable compounds; its decomposition is typically curvilinear, slowing with time (Juma and McGill 1986; Paul 1970). Hunt (1977) has treated all litter as consisting of soft and hard materials, each with a constant decay rate. Paul (1970) and Shields and Paul (1973) estimated separate turnover times for labile and resistant fractions in litter. Clark (1977) studied the rate at which and the route by which nitrogen once taken up by plants in shortgrass moves into litter and soil compartments and from them to new plant growth.

Symbiotic dinitrogen fixation by legumes has commanded wide attention for more than a century (Beringer 1984; Date 1973; Sprent and Bradford 1977). Quantification of dinitrogen fixed in legumes is still not well established due to difficulties encountered in developing valid methods (Mytton 1988; Weaver 1986). The stable isotope N-15 has been used in dinitrogen fixation studies since the first application in agronomy in 1958 by McAuliffe and coworkers. This method, employing N-15 has been considered the only method that provides a valid integrated measurement of N derived from soil and from the atmosphere (Danso 1988; Vose and Victoria 1986). However, a reference crop, which is a nonfixer of dinitrogen is required in the quantification. The balance between soil and fertilizer N which the reference crop obtained relative to a legume, is critical for results obtained, and requires both the legume and reference assimilate soil and fertilizer N in the same ratio (Chalk 1985; Rennie 1982). Barley, wheat and maize are commonly used as

references for legumes because non-fixing isolines are not available except in the case of soybeans (Witty 1983; Witty and Ritz 1984). Therefore, fundamental comparisons of legumes and potential reference crops will test their validity as a reference and also provide a common basic standard for later research and the comparison of results.

The main aim in this project was to develop detailed data of C and N allocation among roots by depth and above ground components of three agronomically important crops for use in developing and testing crop growth models as well as for quantifying dinitrogen fixation in faba bean. The specific objectives of this study were to determine and compare above and below ground dry matter production, and accumulation of C and N among faba bean, canola and barley at four dates in the growing season; to provide further information on differences between the legume (faba bean) and two nonlegumes (canola and barley); and also between the two nonlegumes regarding C and N accumulation. This is the first part of an integrated study of N in soil-plant systems (faba bean, canola, barley and summer fallow).

2.2 Materials and methods

2.2.1 Study area description

The study area was located at the Soil Science plots at Breton (NE-25-47-4W_5), which is 110 km southwest of Edmonton, Alberta, Canada. Soils in this area are dominated by Gray Luvisols and Dark Gray Luvisols (Lindsay *et al.* 1968). The aspect is southwest. Some chemical properties of the soil are presented in Table 2.1. Nitrate and ammonium were extracted with 2M KCl and determined by autoanalyzer. Extractable P was extracted with 0.03M ammonium fluride and 0.015M sulfuric acid. Ratio of 2 : 1 (water: soil) was used for pH determination. Total N was determined an antomated Dumas system (Carlo Erba).

Table 2.1 Some chemical properties of soil from study plots

Depth (cm)	pH	Total N (%)	NO_3-N (ppm)	NH_4-N (ppm)	Total P (%)	Extractable P (ppm)
0-10	6.2	0.19	5.9	6.3	0.06	16
10-20	6.3	0.10	2.3	4.7	0.05	6.0
20-30	6.4	0.07	2.1	4.8	0.04	5.1

2.2.2 Experimental design and plot establishment

This study was a completely randomized block experiment, split within sampling dates. It had four main treatments: faba bean, canola, barley, and summer fallow and was replicated four times. Total field area was 31m x 26m in which each treatment occupied an area of 6.1m x 5.8m. There was a 0.91m open space between each plot. Plots were cultivated to 10-12cm and P was applied at 20 kg P_2O_5 ha^{-1}, followed by seeding on the same day (May 8, 1987). Faba bean (*Vicia faba* (L.) minor. cv. Ackerperle), canola (*Brassica napus* (L.) cv. Westar), and barley (*Hordeum vulgare* (L.) cv. Empress) were seeded at 194, 10, and 89 kg ha^{-1}, respectively. Before seeding faba bean, seeds were mixed in plastic bags with cooking molasess as an adhering agent, then the inoculant (*Rhizobium leguminosarum*) manufactured by the Nitragin Company (Milwaukee, Wisconsin, U.S.A.) was added and further mixed. Due to an unexpected snow-fall in late May, canola was reseeded with a hand-seeder on June 1, 1987. Open-ended steel cylinders were used in this study. The cylinders to contain N-15 were 30cm long and 20cm in diameter. The cylinders were installed after emergence of the crop and were located to cover 2 rows canola and barley or 1 row faba bean in area of uniform emergence. Four cylinders were installed in each plot for each of the four sampling dates conducted during the growing season. The N-15 data will be presented in Chapter 3 and Chapter 4, but all samplings were from within the cylinders.

2.2.3 Sampling and root washing

Samplings were conducted on July 8, July 24, August 19, and September 1 during the growing season, which was 63, 77, 103 and 115 days after seeding barley and faba bean, but 38, 52, 78 and 90 days for canola. At each sampling time, 16 cylinders comprising the four treatments times each of the four replicates were removed from the plots. Above ground portions of each crop were cut at the soil surface, put into a paper bag and oven dried at 65°C for one week before partitioning into various components.

Steel cylinders were transported into the laboratory and subsampled at 0-10, 10-20, and 20-27cm depths. Soil inside the cylinder was first cut vertically into two equal halves and in a direction perpendicular to the crop row. One of them was taken out, cut into the three depths, and put into plastic bags for root washing later. The remaining half inside the cylinder was further cut into two quarters, one of which was cut into three depths and put in aluminium trays to air-dry for soil total N and N-15 abundance analyses. The other was divided into three depths and stored in a cooler at 4°C for microbial biomass, and mineral

NH_4^+ and NO_3^- analyses.

Root washing was conducted with a root-washer constructed by the Machine Shop, University of Alberta following a design from the Alberta Environmental Centre, which is a modification of a design by Cahoon and Morton (1960), McKell *et al.*. (1961) and Welbank and Williams (1968). An air pressure pump and tap water were attached to the root-washer. The water pressure at the inlet was 50psi (about 350 Kpa) with an air pressure of 100psi for the whole system (6 units). Separation of root from soil samples was actually by flotation (Cahoon and Morton 1960; Haas 1958; McKell *et al.* 1961). Mixed fresh roots and dead plant debris were collected and further separated (Hass 1958) by floatation and tweezers. In our study, separation was by means of water flotation to separate clay material from living and dead material of crops, and tweezers to separate the two components: fresh roots and organic debris. After differentiation, both fractions were oven dried at 65°C for 1-2 weeks, and then weighed.

2.2.4 Plant material partitioning

All above-ground plant materials were dried and weighed followed by fractionation. For the 1st sampling, faba bean and canola were partitioned into stems and leaves; and barley into stems, sheaths, dead-leaves, and live-leaves. If a visible spot of green color remained, leaves were classified as live; otherwise they were classified as dead-leaves. On the 2nd sampling, faba bean and canola were again partionated into stems and leaves; while barley was separated into stems, sheaths, dead-leaves, live-leaves, and heads. On the 3rd sampling, faba bean and canola were fractionated into leaves, stems, shells, and seeds; barley into stems, sheaths, dead-leaves, live-leaves, seeds, and husks. The term 'husks' was used to designate pod components after seeds were removed for faba bean and canola, whereas it was the head components after seeds were removed from barley. On the last sampling, canola was stems, seeds and husks; faba bean was stems, leaves, seeds and husks; and barley was dead-leaves, stems, sheaths, seeds and husks.

2.2.5 Analyses

Partitioned samples of crop materials were either ground in a Wiley mill (Model 5KH33GG2, Arthur H. Thomas Co., Philadelphia, U.S.A.), such as faba bean roots of 0-10cm depth, faba bean stems, or in a coffee mill (Braun Canada Ltd., Type KSM2, Spain), such as leaves, before final pulverizing in a vibrating-ball mill (Retsch, Type MM2, Brinkmann Instruments Co., Ontario, Canda), which is a special requirement for ^{15}N

sample preparation. Soil samples were ground and passed through a 2mm mesh first, then subsamples were taken for further grinding in the ball-vibrating mill. During grinding on the vibrating mill, isopropyl alcohol was used to clean the grinder between samples of different treatments after vacuum cleaning (Binkley *et al.* 1985). Samples were analyzed for organic C content using a Leco Carbon Determinator CR-12 by dry combustion (McKeague 1978). Total N was determined by automated Dumas combustion using a Carlo Erba C, N, S analyzer.

2.2.6 Calculations

Total dry matter of crop tops was the original weight data obtained after drying in an oven but before fractionation, while total C and N accumulated in crop tops was the summation of averaged C and N in various components calculated from C or N content and weight of each of the fractions. Root dry matter, and accumulated C and N were the summation over the three depths for each of the treatments.

2.2.7 Statistical analyses

Analysis of variance was carried out using program P2V of BMDP statistical software (Dixon 1983). Multiple comparisons with Newman-Student-Keuls (Sokal and Rohl 1981) were conducted after a significant effect was found for treatments. Regression was done for dry matter production over time. The Chi-square test was conducted on data of root distribution over three depths.

2.3 Results

2.3.1 Above ground components

2.3.1.1 Total dry matter and N accumulated in crop tops

Crop species, sampling dates, and interactions of crop and sampling date effects were significant ($P < 0.01$) on dry matter production (DMP) of crop tops. Further examination has shown that no significant differences between canola and barley on DMP of the four sampling dates although DMP had increased significantly from 108.8 to 349.6 g m^{-2}, and 138.6 to 349.0 g m^{-2} for canola and barley from the 1st sampling (July 8) to the last sampling (September 1), respectively (Fig. 2.1a). By the last sampling (September 1), canola and barley had fully matured but faba beans were still in the grain-filling stage. There were no significant differences on DMP of faba bean between the first two or the last

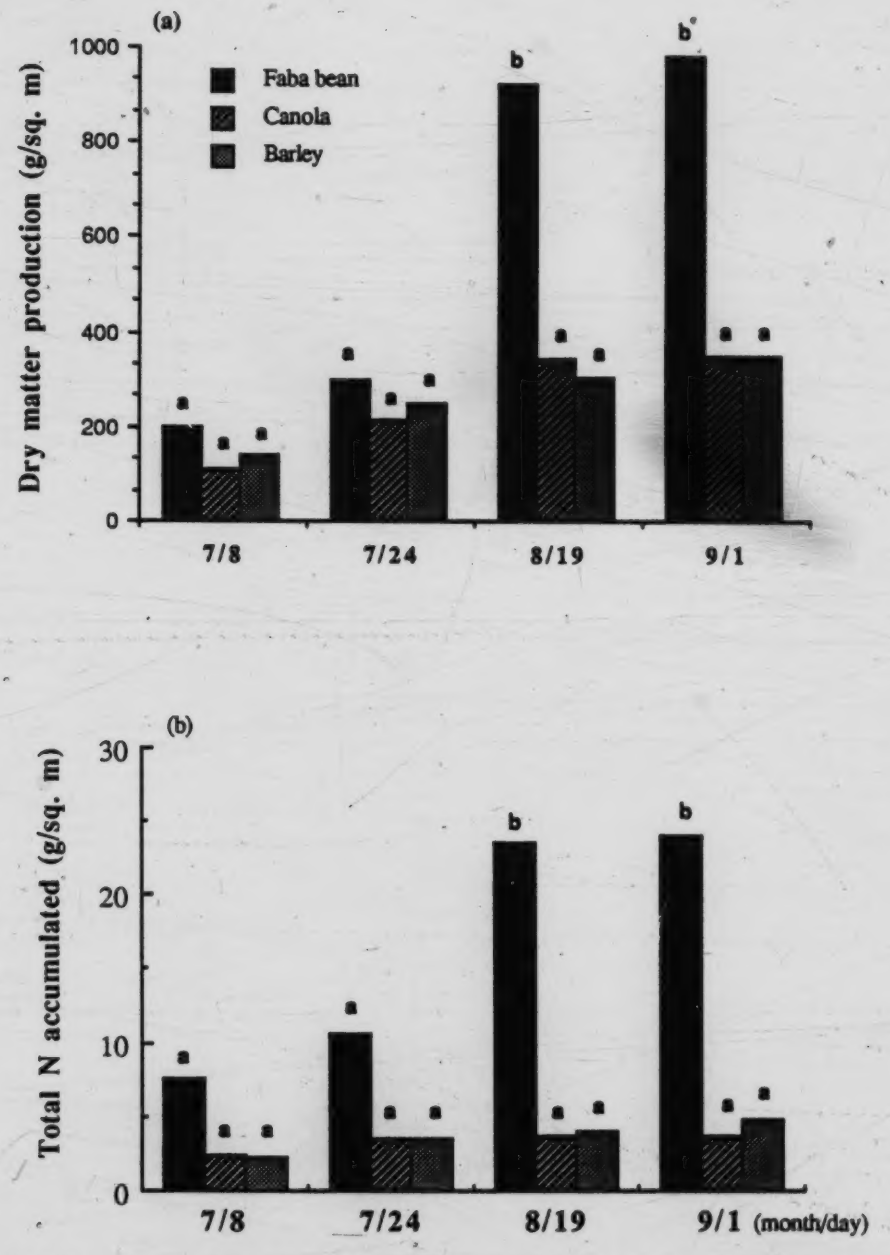

Fig. 2.1 Dry matter production and N accumulated in crop tops.

a-b means followed by the same letters do not differ significantly on one date ($p < 0.01$)

two samplings, but a significant difference between the first two samplings and the last two samplings. Faba bean DMP had increased from 199.4 to 981.8 g m^{-2} from the 1st to the last sampling. Actually, dry matter of faba beans had increased continuously across the four samplings conducted over the growing season, but a significant increase was observed only between the 2nd and 3rd samplings when the faba beans began flowering. During this period, DMP of the faba beans had increased from 301g m^{-2} at the 2nd sampling to 919 g m^{-2} at the 3rd sampling; during the same period, the crop growth rate was 23.8 g m^{-2} day^{-1}. Similarly, DMPs of canola and barley had increased from 215 to 343 g m^{-2}, and 250 to 304 g m^{-2} for the same time period, respectively. The crop growth rate was 4.9 and 2.1 g m^{-2} day^{-1} for canola and barley. DMP of faba bean was about 3 times that of canola or barley on the 3rd sampling. Crop growth rate of faba bean reached 5 to 11 times that of canola and barley between the 2nd and 3rd sampling.

Crop species, sampling date, and interactions of crop and sampling date effects were significant ($p < 0.01$) for N accumulation in crop tops. N accumulation in faba bean tops was higher than in canola and barley on the 3rd and 4th sampling dates (Fig. 2.1b). Differences in N accumulated in canola and barley were not significant between any of the four sampling dates. N concentrations in dry matter were different , declining from 2.2-1.1% and 1.7-1.3% for canola and barley, respectively over the four sampling dates. N content in faba bean was two times that in canola and barley except for the 1st sampling, which was 1.7 and 2.3 times that in canola or barley, respectively. For the first sampling, N accumulated in tops was 7.5, 2.4, 2.3 g m^{-2} for faba bean, canola and barley, respectively. From the 2nd to 3rd sampling, N accumulated in faba beans increased by 13 g m^{-2} (Fig. 2.1b) while the N increment to barley and canola was only 0.48 and 0.14 g m^{-2}, respectively. N accumulated in canola and barley varied between 2.4-3.7 and 2.3-4.8 g N m^{-2} compared to 7.5-24.1 g N m^{-2} in faba beans over the four samplings. The N accumulated in faba bean tops was 6 times that in canola and 5 times that in barley on the final sampling.

2.3.1.2 Fractionation of crop tops

Fractionation of plant dry matter provided specific information on the contribution of dry matter from various components. Dry matter production of various faba bean components followed the sequence, stems > leaves > husks > seeds on the two last samplings, when faba beans were in the grain-filling stage (Fig. 2.2a). Dry matter of faba bean leaves was 2.4 and 1.2 times that in stems on the first two samplings, but the rapid increase of stems resulted in this out-weighing leaves on the 3rd and 4th samplings (Fig.

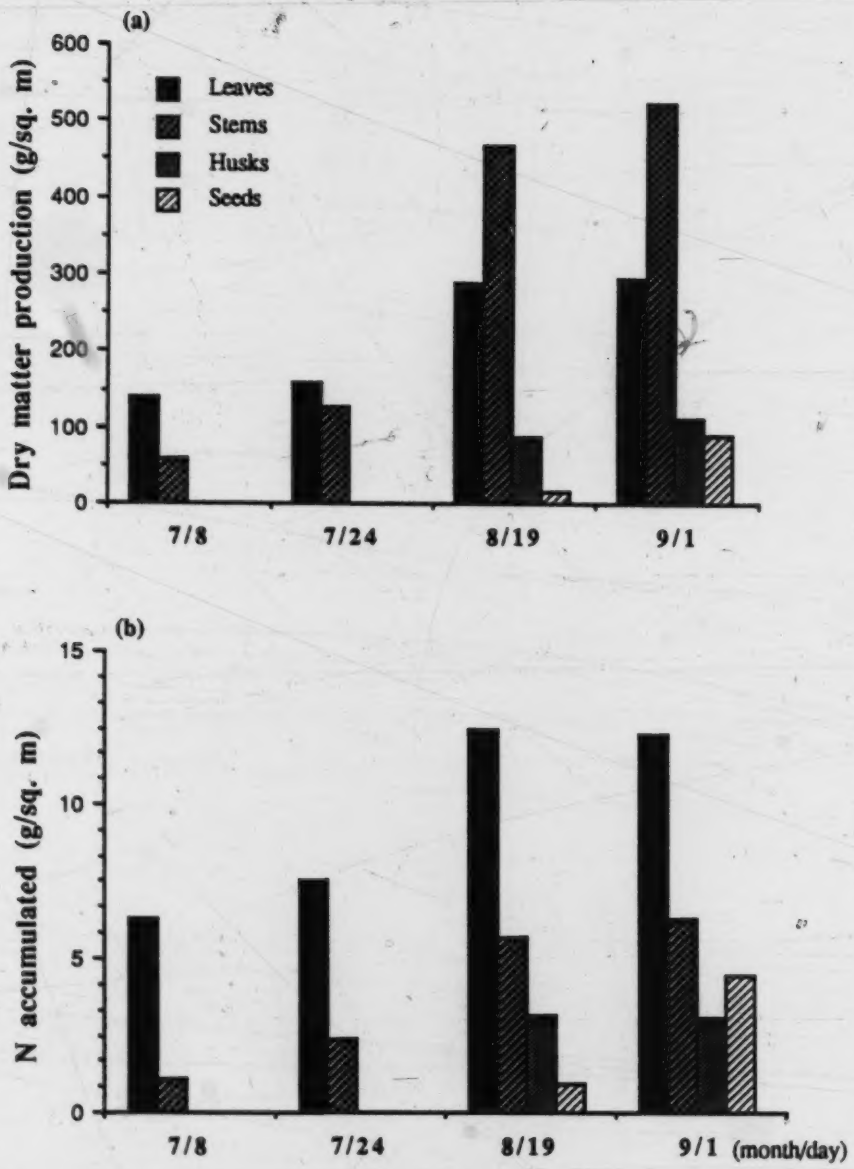

Fig. 2.2 Dry matter production and N accumulation in faba bean tops

2.2a). Dry matter of faba bean stems became 1.6 and 1.8 times that in leaves at the 3rd and 4th samplings. During the sampling intervals, faba bean leaves increased by 13, 84 and 3% compared to 118, 266 and 12% in stems. Seeds were 16 g m^{-2} on the 3rd sampling, and reached 88.9 g m^{-2} on the last sampling with a crop growth rate of 6.1 g m^{-2} day^{-1}, but it was not fully matured even on the last sampling (September 1). N accumulation in faba bean leaves was much higher than in any of the other components; and followed leaves > stems > seeds > husks on the 4th sampling (Fig. 2.2b). At the 3rd sampling, faba bean N had accumulated mostly in leaves, 13 g N m^{-2} compared to 6 g N m^{-2} in stems. N in stems did not increase from the 3rd to 4th sampling, but N in seeds increased 4 times (Fig. 2.2b). The N contents in leaves and stems were 4.6-4.2% and 1.9-1.2 decreasing as they grew. By September 1, the quantity of N accumulated in leaves and stems was 12.3 and 6.27 g m^{-2}. Between the 3rd and 4th samplings, N in husks was relatively constant (3.1-3.2 g m^{-2}), but increased at a rate of 0.3 g m^{-2} day^{-1} in seeds to yield a final seed N content of 4.5 g m^{-2}.

With canola, DMP was increased mostly by stems and seeds while leaves decreased continuously over the four samplings in the growing season (Fig. 2.3a). Leaves and stems accounted for 58 and 42% of the total dry matter on the 1st sampling. Between the 1st and 2nd samplings, DMP increased 11.0 and 206% for leaves and stems, respectively. Stems continued to increase whereas leaves had decreased by the 3rd sampling. Generally, husks increased slightly from the 3rd to 4th sampling; varying between 89.0 and 94.5 g m^{-2}. Seeds increased rapidly from 29.1 at the 3rd sampling to 54.3 g m^{-2} on the last sampling, accounting for 16% of the total dry matter. N accumulated in canola leaves was higher than in stems for the first 2 samplings though stems accounted for 67% of the dry matter on the 2nd sampling. On the 1st and 2nd samplings, N accumulated in leaves accounted for 71 and 60% of the total N in canola tops (Fig. 2.3b). As canola developed from vegetative to regenerative growth, N in leaves decreased and by the 3rd sampling accounted for only 20% of the quantity present at the 2nd sampling. By the final sampling, N in seeds alone accounted for 76% of the total N accumulated in canola tops.

DMP of barley became dominated by seed production. Seeds accounted for 46 and 49% of the total dry matter accumulated as barley tops on the 3rd and 4th samplings (Fig. 2.4a). Live-leaves decreased from 31.2 g m^{-2} on the 1st sampling to 0.6 g m^{-2} on the 3rd sampling, whereas stems increased from 26 to 59 g m^{-2} over the same interval. Dry matter of dead-leaves and sheaths changed only slightly over time. Seeds increased dramatically after the 2nd sampling; reaching 140.1 g m^{-2} at the 3rd sampling and continuing to 169.4 g

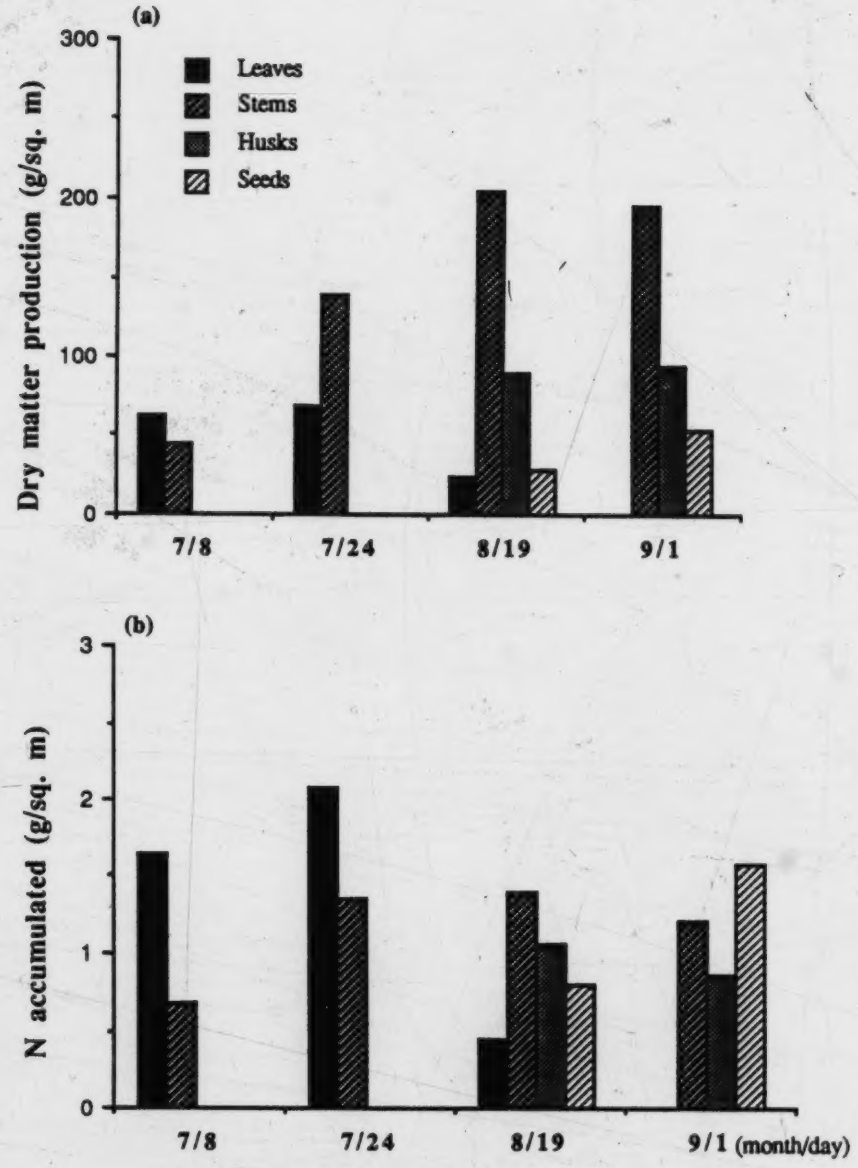

Fig. 2.3 Dry matter production and N accumulated in canola tops

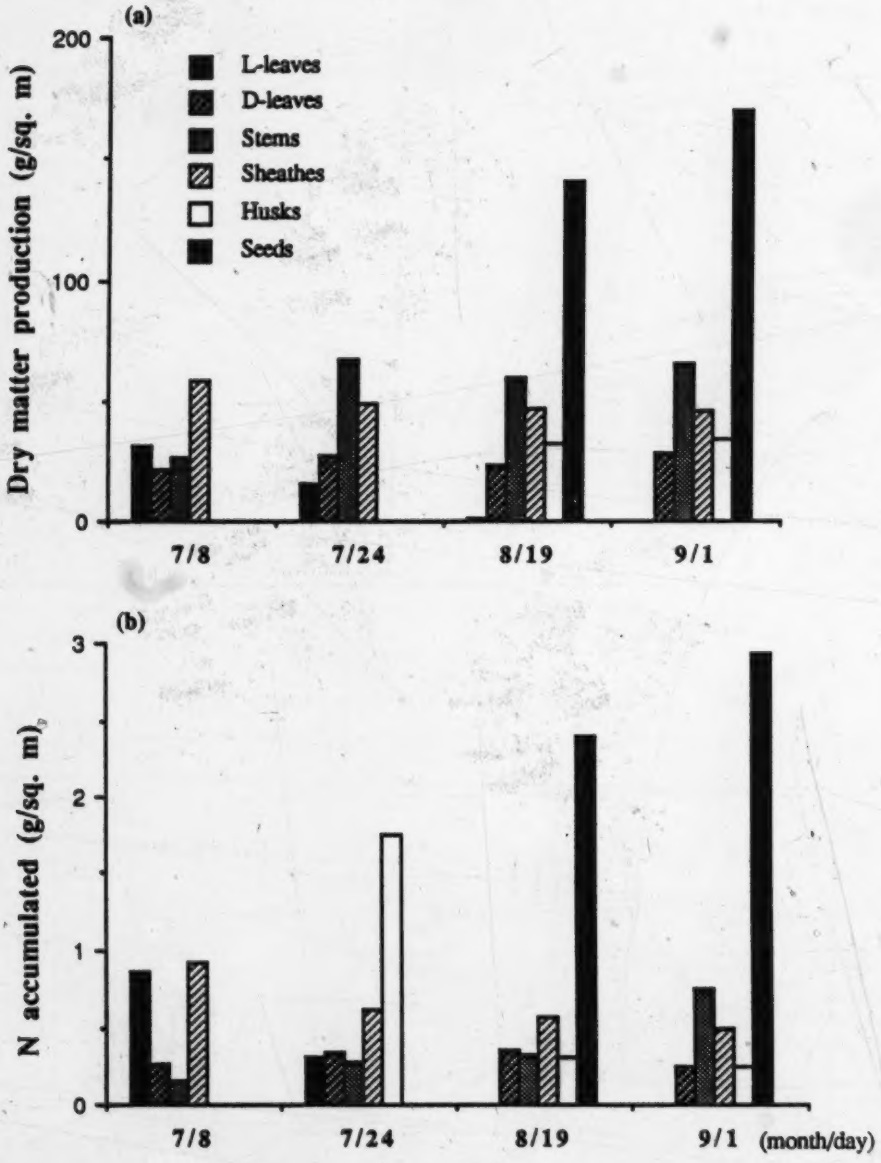

Fig. 2.4 Dry matter production and N accumulated in barley tops

m^{-2} by the last sampling. The crop growth rate during this period was 2.4 g m^{-2} day^{-1}. N accumulation in various components of barley paralleled the trend in DMP, with the exception that N accumulation in seeds was even more pronounced (Fig. 2.4b). On the 1st sampling, N accumulated in live-leaves and sheaths was 39 and 42%, respectively of the total N in crop tops. By the 2nd sampling, husks were the largest single component, containing 53% of above ground N in the crop. Seed N dominated on both the 3rd and 4th samplings, and accounted for 61 and 63% respectively of the total N in barley tops.

2.3.2 Below-ground components

2.3.2.1 Total dry matter and N accumulated in crop roots

Crop species, sampling dates, depth, and interactions of crop and depth effects were significant ($P < 0.01$) on dry matter production of crop roots. Interaction between sampling date and depth was significant ($p < 0.05$) whereas interaction between crop and sampling date was not significant ($p < 0.1$). Interactions among crop, sampling and depth effects were not significant ($p > 0.1$). Dry matter production of crop roots totalled over the three depths followed a pattern similar to crop tops; root dry matter of faba bean exhibited a rapid increase between the 2nd and 3th sampling (Fig. 2.5a) and was greater than either canola or barley over all four samplings. Faba bean roots were 2.5-3.9 times that of canola and 1.8-3.3 that of barley. Root dry matter of canola and barley varied only from 41.7 to 75.9 g m^{-2} and 50.0 to 88.5 g m^{-2}, respectively; whereas it more than doubled from 125.1 to 295.6 g m^{-2} for faba bean between the 1st to the last samplings. Between the 2nd and 3rd sampling, the interval of most rapid growth, growth rate of crop roots was 4.8 g m^{-2} day^{-1} in faba beans, but only 1.3 g m^{-2} day^{-1} in canola and 0.5 g m^{-2} day^{-1} in barley.

Crop, sampling, depth, interaction of crop and depth, and interaction of sampling date and depth effects were significant effects ($p < 0.01$) on N accumulation. Interaction of crop and sampling date effect is significant ($p < 0.05$), but interaction of crop, sampling, and depth effects were not significant ($p > 0.1$) on N accumulated. N accumulation in roots of faba bean within 27cm was significantly higher than in canola or barley on all dates, becoming more pronouced as the crops grew (Fig. 2.5b). N accumulated between the 1st and last sampling ranged between 2.9-5.6 for faba bean; 0.75-0.94 for canola; and 0.59-1.15 g N m^{-2} for barley.

2.3.2.2 Crop roots in three depths

Root dry matter did not vary significantly between 10-20 and 20-27cm on the four

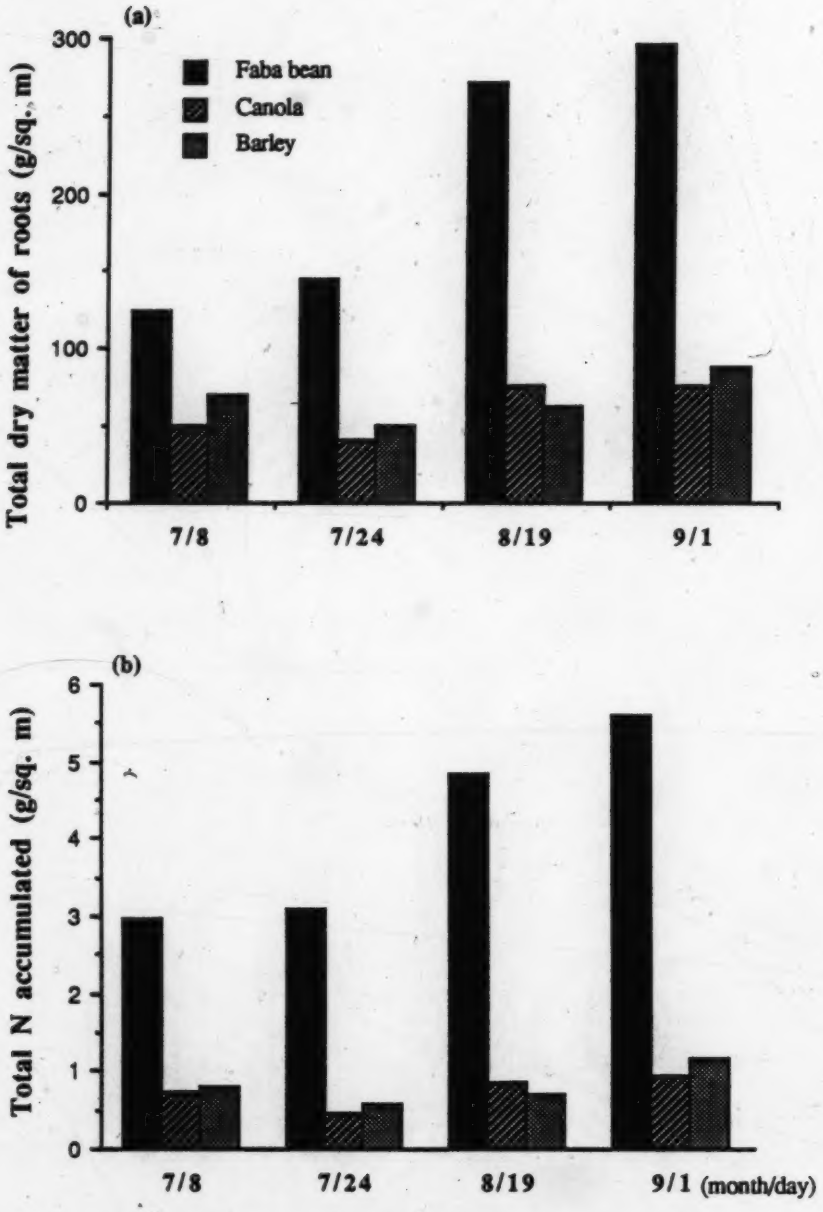

Fig. 2.5 Total dry matter and N accumulated in crop roots

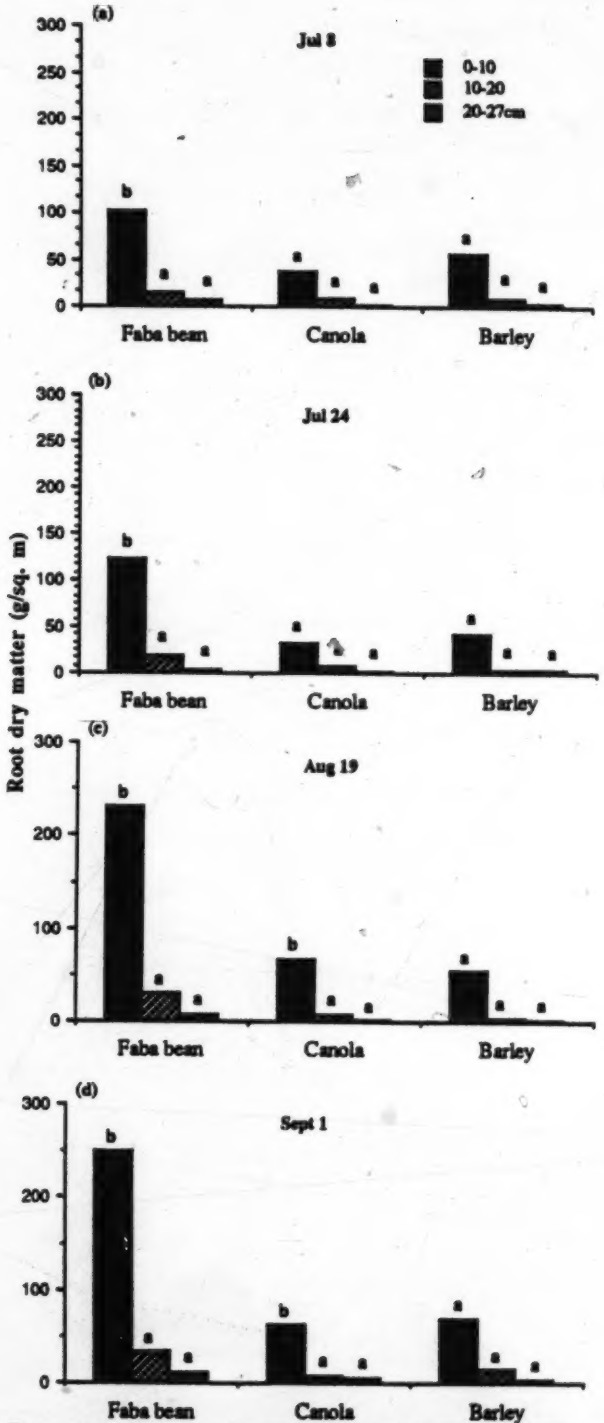

Fig. 2.6 Dry matter production of crop roots over one growing season
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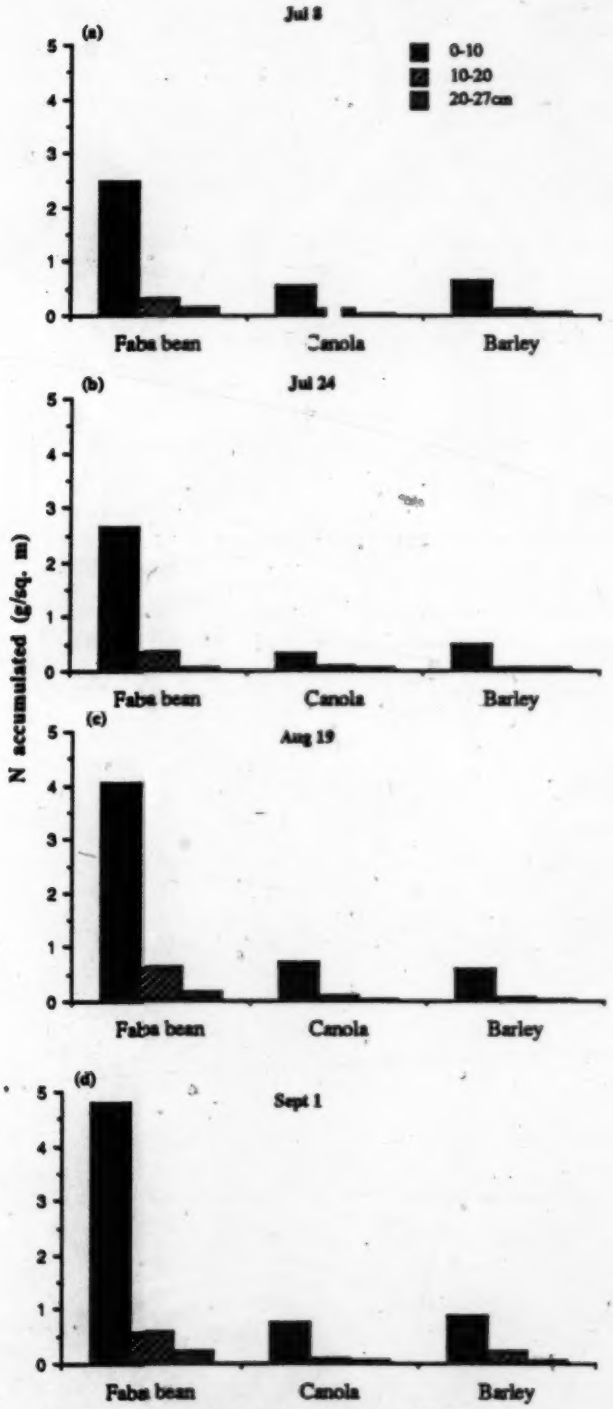

Fig. 2.7 N accumulation in crop roots (0-27cm).

samplings conducted. Faba bean roots had the highest dry matter over the four sampling dates through the three depths (Fig. 2.6). Root distribution in 0-10, 10-20 and 20-27cm depths on average over the four samplings were 85, 12 and 3% for faba bean; 82, 13 and 5% for canola; and 83, 13 and 4% for barley. It was observed that soil became denser below 15-17cm; physical restriction was visible for root development and penetration into this layer. These dramatic decreases in root dry matter may be associated with soil physical properties on one hand; and crop root morphological characteristics on the others. N accumulated in root of 0-10, 10-20 and 20-27cm followed the pattern of dry matter distribution; decreasing with depth, and with more N in faba bean than canola or barley (Fig. 2.7). N accumulated in 0-10, 10-20 and 20-27cm varied between 2.5-4.8, 0.34-0.59 and 0.07-0.24 g N m^{-2} for faba bean; 0.32-0.75, 0.10-0.12 and 0.04-0.08 g N m^{-2} for canola; and 0.48-0.87, 0.05-0.22 and 0.04-0.06 g N m^{-2} for barley. By September 1, percentage of total N accumulated in the roots over the three depths was 85, 10 and 5% in faba bean; 80, 12 and 8% in canola; and 76, 19 and 5% in barley.

2.4 Discussion

2.4.1 Above ground components

Dry matter accumulation in crop tops over time (days) was fitted with a simple linear model, which is $Y = -69.039 + 3.6847X$ ($r^2=0.924$) for barley; $Y = -50.538 + 4.7217X$ ($r^2=0.948$) for canola; and $Y = -902.87 + 16.794X$ ($r^2=0.959$) for faba bean. The dramatic dry matter production in faba beans from July 24 to August 19 coincided with growth transition from vegetative to generative stage; the crop growth rate during this period was 23.8 g m^{-2} day^{-1}. Richards and Soper (1982) observed the maximum rate of dry matter accumulation and N uptake commenced at flowering and continued up to the mid pod-filling growth stage in Manitoba.

N accumulated in the three crop was fitted to a simple linear model mainly and it is $Y = -15.439 + 0.35647X$ ($r^2=0.956$) for faba bean and $Y = -6.7499\times10^{-2} + 4.1313\times10^{-2}X$ ($r^2=0.924$) for barley. A polynomial model was used for N accumulated in canola and it is $Y = -2.4040 + 0.17177X - 1.1701\times10^{-3}X^2$ ($r^2=0.931$). Legume (faba bean) and non-legumes (canola and barley) differed significantly in their pattern of dry matter accumulation in tops. A maximum of DMP in faba bean is predicted near the last sampling (September 1), whereas canola and barley reached their maximum dry matter accumulation between August 19 and September 1. Maximum weight of faba bean stems was reached on August 19, amounting to 522.0 g m^{-2}. Similar results have been reported (Hill-

Cittingham and Lloyd-Jones 1980;, Ishag 1973a; 1973b; Patriquin *et al.* 1981; Rennie and Dubetz 1986; and Richards and Soper 1982). Richards and Soper (1982) reported that barley and uninoculated faba beans which received no fertilizer N contained similar quantities of shoot N at Altona in manitoba, but considerable differences in N uptake by N fertilized barley and uninoculated faba beans occured at Seven Sisters in Manitoba during a dinitrogen fixation study of inoculation effects. N accumulated in faba bean tops which were well-inoculated in our field study at Breton plots, was higher than that in canola or barley, being 24.1 g N m^{-2} in the former, and 3.7 and 4.8 g N m^{-2} in the latter, respectively (Fig 2.1).

Dry matter in canola was lower than in faba bean by 3 fold for DMP and N accumulation was lower by 6 fold (Fig. 2.1). Seed yield of canola was 54.3 g m^{-2}, which was in the lower part of the control compared to results reported by Malhi *et al.* (1988). This may be due to reseeding necessitated by snow storm in late May and the shortening of the growing season, or constraints imposed by steel cylinders, or low fertility of this soil. Steel cylinders were employed in this study and the constraints imposed may affect root growth and the exploration area of the root system.

Barley DMP was similar to the results reported by Hansson *et al.* (1987). Barley stems increased by 75% from the 1st to the 2nd sampling. Stems may accumulate a high proportion of leaf photosynthate and also serve as a source of carbohydrates and nitrogenous compounds to be mobilized and translocated to the kernels during grain-filling (McLelland 1984; Wych *et al.* 1985). N accumulated in barley amounted to 4.76 g m^{-2} and is in agreement with results from Seven Sister in Manitoba (Richards and Soper 1982). There was no significant difference between N accumulated in canola and barley. Therefore, on this soil, under these conditions, both barley and canola have similar abilities to exploit soil N. They therefore may serve equally well as controls for quantification of dinitrogen fixation.

C assimilation in crop tops had similar patterns as that for total dry matter production in these three crops, percentage organic C in tops varied between 39.84-44.43% in faba bean; 43.44-46.66% in canola and 42.64-44.62% in barley.

Carbon to nitrogen ratio of faba bean was 2-3 fold lower than in either canola or barley on any of the four samplings. For all three crops, C/N ratio increased consistently on the four samplings. Significant increases of C/N ratios were observed for faba bean and canola between July 24 and August 19; percentages were 43% and 65%, respectively. C/N

ratio ranged within 11.3-18.1 for faba bean; 20.2-44.32 for canola and 26.7-34.4 for barley. At the last sampling, C/N ratio was 18.1, 44.32, 34.4 for faba bean, canola and barley, respectively. On September 1, the C/N ratio of seed components of faba bean was 16.1 at the stage of grain filling; for canola it was 63.3; and 43.7 for barley. The largest N component of nonharvestable faba bean was leaves (57%) which had a C/N ratio of 10.1. For canola, however, the largest nonharvestable N component was stems (58%) with a C/N ratio of 73.1; but for barley, stems and sheaths totalled 72% of the nonharvestable N with C/N ratios of 38.0 and 40.9, respectively.

It is very important that the main value of a legume is a long-term one in maintaining soil organic N concentration to ensure adequate delivery of N to subsequent cereal crops in rotation (Ladd *et al.* 1981). Wheat assimilated 20.2-27.8% of its N from legume residue , which was applied at 48.41 kg N ha^{-1} as *Medicago littoralis* (Ladd *et al.* 1983). The lower C/N ratio and the structure of material in legumes lead to easy access for microbial attack. The chemical composition of plant materials, such as nitrogen content, lignin and carbohydrate are important in considering decomposition processes (Herman *et al.* 1977). Equations have been developed using C/N ratio to describe decomposition (Paul and van Veen 1978) or to partition residues into various components with diverse behaviour in soil (Hunt 1977; McGill *et al.* 1981). The architecture of molecular arrangement and intracellular associations may take precedence over links between monomers, or characteristics of isolated organic and mineral soil components (Juma and McGill 1986; McGill and Myers 1987). To the extent that C/N ratio influences N release from crop residues, it appears that although faba bean stems may decompose more slowly, the leaf component is larger and its N would be expected to be released rapidly. In canola, on the other hand, much of the N is present in stems with a high C/N ratio and would therefore likely be of limited availability to immediately succeeding crops. Barley residues appear to be intermediate.

2.4.2 Below ground components

Root dry matter was concentrated in the upper 0-10cm, which accounted for 82-83% in canola and barley, and 85% in faba bean. Chi-square analysis of data for root distribution by depth indicated that the patterns of root distribution of the three crops were not different. The proportion of roots in each of the three depths followed the same trend. Although results may be a coincidence of the soil physical condition in this Gray Luvisol, the same patterns of gross vertical root distribution in soil of this study provides evidence to support the assumptions in the N-15 isotope dilution assay of dinitrogen fixation

(McAuliffe *et al.* 1958). These results further suggested that on the basis of root distribution patterns on this soil, both canola and barley exploit similar soil regions and in equal proportions. The absolute quantity of root material varied, but the proportions of soil N received from each depth would appear to be the same for all three crops. These two crops are also not different from faba bean with regard to root distribution. They should, therefore, both be equally suited for use as a reference crop for quantification of dinitrogen fixation under the conditions used here.

At harvesting, straw is often taken out of the field, or burned in some cases and then roots provide the major organic matter input to the soil. Root C accounted for between 20-35%, 15-30% and 25-30% of the total C assimilated by faba bean, canola and barley, respectively. Faba bean roots produced as much or more dry matter (296 g m^{-2}) than was present in all nonharvestable above ground materials of canola (294 g m^{-2}) or barley (173 g m^{-2}). The nonharvestable tops/roots ratio was 3.14 for faba bean; 3.84 for canola; and 1.96 for barley on September 1. Furthermore, with respect to N, faba bean roots contained 5.61 g m^{-2} which was 1.86 times as much as was in all nonharvestable tops plus roots of canola and 1.95 times that in barley. Consequently, even if all the N in faba bean tops is removed, which is not likely, the large root mass remaining still returns almost double the N in canola or barley to soil (perhaps adds if N fixation is sufficient) than would be returned if all shoots plus roots of barley or canola remained in the field. These crops were grown without added N but fertilizer would have to roughly double straw and root N contents to bring them up to faba beans.

Hansson *et al.* (1987) obtained relatively higher root dry matter and N accumulation in barley, and the deviation from our results is thought to be caused by differences between soils used and sampling methods chosen. During sampling, they used four cores (diameter 7cm), two within sowing rows and two between sowing rows, which were divided into 0-10, 10-27cm and the sand layer (Hansson *et al.* 1987); whereas steel cylinders (diameter 20cm) were taken for subsampling and root-washing in this study. Yamauchi *et al.* (1987b) classified barley roots as "scattered type of root system" because roots in this group were well developed and almost all of the nodal roots ran obliquely in the soil profile. In another study on soybean, Kono *et al.* (1987b) observed that lateral roots, which accounted for over 98% of total root surface area as well as total root length, were essential to the enlargement of root system. A large volume of soil (0-27cm) was available for barley development (Hansson *et al.* 1987), but in this study steel cylinders and soil physical properties may have constrained development. Furthermore, no fertilizer N

was applied on our study site which likely reduced the vigor of the crop and extent of exploration of soil by roots.

Hansson and Andren (1987) found greater root densities and deeper root penetration in fertilized barley using mini-rhizotrons to study root structure. In another report, Hansson *et al.* (1987) found the fertilizer N effect was more pronouced on barley tops than on root growth. This suggested that C allocation of barley in fertilized soil favored tops, but was shifted toward roots when the soil was not fertilized. In comparing root length and dry weight of six green manure legumes, Kirchmann (1988) found that white clover (*Trifolium repens* L.) and red clover (*T. pratense* L.) accumulated larger amounts of N in their roots and there was an unequal distribution of nitrogen between shoots and roots. Therefore, the architectural concept in agroecosystems may be applied here to understand relationships between root growth and nutrient availability in soil (McGill and Myers 1987). In this Gray Luvisol where our experiment was set up, there is a transition from loam to heavy clay with high dens..y and massive structure starting around 15-17cm downward (Canda Soil Survey Committee 1978), which is a physical barrier for crop root development. This was observed during subsampling of soil in the cylinders. Hansson *et al.* (1987) also pointed out the impact of soil structure on root penetration and 90-97% of the roots were in 0-27cm of the loam they used compared to only 4-5% in the sandy layer. Soil structure affects root growth, but crop roots can alter soil structure in return by using appropriate crops, which can penetrate and improve soil physical and chemical properties.

Root development of faba bean was synchronized with tops, but the synchronization was not obvious in canola or barley.

In conclusion, dry matter production and N accumulation of faba bean differed significantly from canola and barley for above and below ground components, but there was no difference between canola and barley. Root distribution patterns of these three crops were not different. Therefore, barley and canola were equally valid reference crops for quantification of dinitrogen fixation in faba bean. N in roots of faba bean was as much as that in tops plus roots of barley and canola, so fate of faba bean residues, decomposition of faba bean, and nutrient dynamics are important in understanding plant-soil system and managing agricultural ecosystem.

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CHAPTER 3. ALLOCATION OF N-15 AMONG ABOVE AND BELOW GROUND COMPONENTS OF FABA BEAN, CANOLA AND BARLEY AND QUANTIFICATION OF SYMBIOTICALLY FIXED N BY FABA BEAN ON A GRAY LUVISOL[2]

3.1 Introduction

Biological dinitrogen fixation has commanded the attention of scientists concerned with plant mineral nutrition and soil management for more than 100 years (Date 1973; Dixon and Wheeler 1986; Faust 1982; Havelka *et al.* 1982; Ladd *et al.* 1981; Ladd *et al.* 1983; Ladd *et al.* 1986; McGill and Christie 1983; Muller 1988; Muller and Sundman 1988). Public concern about environmental quality together with increases in the price of fuel which in turn influences the cost of industrial N production, and concerns about soil conservation and crop diversification have stimulated interest in biological dinitrogen fixation, with most recent emphasis on pulse crops (Patriquin *et al.* 1981; Sprent 1986). Green manures and legumes are being examined with regard to their potential role in present day agriculture (McGill 1982; Patriquin *et al.* 1981). Assessing the quantities of N fixed, however, remains a problem.

Since the first application of N-15 in agricultural research (McAuliffe *et al.* 1958), methods to quantify dinitrogen fixation have been challenged by the search for reliable methods. Several methods are available for quantifying biological nitrogen fixation, but each has its own problems (LaRue and Patterson 1981). The total N balance method (TDM), N-15 dilution, and acetylene reduction (AR), may yield different results with different approaches in methodology (Smith and Hume 1987). Acetylene reduction (AR) is useful for its high sensitivity and for its ability to provide short term information about biological nitrogen fixation and activity of nitrogenase enzyme.

N-15 dilution and TDM both provides integrates long term data. Of these, only N-15 dilution can distinguish the source of N in legumes and thereby provide a direct, integrated measurement of fixation. Such an approach to assessing dinitrogen fixation, however requires an appropriate non-fixing reference crop. For the experiment to be valid, there must be no N transfer from the dinitrogen fixing system to the non-fixing system (Ledgard *et al.* 1985d; McAuliffe *et al.* 1958). Reasonable results are not available to

[2]A version of this chapter will be submitted for publication by Gu, J.-D. and W.B. McGill.

determine the most suitable species to use as a reference plant when using N-15 dilution methods, nor to assess the efficiency of the method under a range of soil conditions. For example, in pots low in N, 87% of the plant N was derived from fixation (A-value method), but fertilizer addition decreased fixation without increasing total N (LaRue and Patterson 1981). Ledgard *et al.* (1985c) observed that the natural abundance of N-15 in clover (*Trifolium subterraneum* L. cv. Woogeneiiup) roots was significantly higher than in the shoots, but there was no significant difference between shoots and roots of ryegrass (*Lolium rigidum* Gaudin cv. Wiimmera).

Nitrogen-15 isotope techniques have been applied widely to N related research, including symbiotic dinitrogen fixation studies (Boddy *et al.* 1983; Butler and Ladd 1985; McAuliffe *et al.* 1958; Rennie and Dubetz 1986; Witty 1983), mineralization and immobilization of nitrogen through microorganisms (Juma and Paul 1981), and N turnover in reclamation of mined sites (Fyles and McGill 1987). The basic principle involved in nitrogen isotope dilution methods was elucidated by Fried and Dean (1952) and has been widely used to quantify dinitrogen fixation in legumes (Fried and Broeshart 1975; Fried *et al.* 1983; Goh and Edmeades 1978; Rennie 1982; Witty 1983). A non-legume crop is usually used as a reference plant in the N-15 dilution technique for field quantification (Fried *et al.* 1983) although an ineffectively inoculated nodulating line may be preferable (Rennie 1982). The difference in labelled nitrogen between reference plant and dinitrogrn fixing plant is used to calculate the dinitrogen fixed (Vallis *et al* 1967). Consequently, the accuracy of the results may be influenced by morphological and physiological differences between root systems of the reference plants and the legumes (Bergersen and Turner 1983; McAuliffe *et al.* 1958; Vallis and Henzens 1977).

The primary assumptions by McAuliffe *et al.* (1958) were that reference plant and legume absorb N-15 and nonlabelled mineral N at the same ratio and there was no transfer of N from legume to associated plants. The reference plant and the legume must absorb added N-15 and soil N in the same ratio (R). The added N-15 is usually in the form of mineral N and Ledgard *et al.* (1985a; 1985b; 1985c) developed a method to calculate R directly for the legume and reference. Meanwhile, Chalk *et al.* (1983), Witty (1983), Witty and Ritz (1984) recommended N-15 incorporation in organic form, or accompanying inorganic N with a carbon source to stablize the mineralization process of N during a growing season. Such techniques would reduce differences in R due to changes in relative availability of soil and N-15 over the growing season which could yield differences in R between reference plant and legume. A technique by Vallis *et al.* (1967) involving multiple

small applications of highly labelled N is frequently used. Problems, such as the discriminative uptake between nitrate and ammonium, application times, amount to apply, and the forms of nitrogen used, have been discussed in recent review (Chalk 1985; Danso 1986).

Faba bean (*Vicia faba* (L) minor.) is one of the most important grain and forage legumes widely distributed over the world. In Asia, China has the largest production of faba bean (Tao 1980). Faba bean has drawn wide attention in Western Canada due to its agronomic performance and high protein content (Candlish and Clark 1975; Evans *et al.* 1972). In 1978, about 5000 hectares, of which 1500 hectares were irrigated, were grown in Alberta (Krogman *et al.* 1980). It is an important source of dietary protein especially in rural communities in developing and less developed countries for human consumption (Saxena and Stewart 1983). Amount of nitrogen reported to be fixed by faba bean ranges from 54 to 146 kg ha^{-1} in Western Canada (Dean and Clark 1977; Rice 1976; Richards and Soper 1979). As much as 209 kg N ha^{-1} has also been reported (Zapata *et al.* 1987). Faba beans fixed a significant amount of N in intercropped system with barley, but there was no evidence of N transfer from faba bean to barley in that system (Danso *et al.* 1987).

In the Chapter 2, it was shown that when grown in metal cylinders on a Luvisolic soil at Breton, the gross root vertical distributions of barley (*Hordeum vulgare* (L.) cv. Empress), Canola (*Brassica napus* (L.) cv. Westar) and faba bean (*Vicia faba* (L.) minor. cv. Ackerperle) were not different. Under such conditions, and with several small additions of highly N-15 labelled fertilizer, the N-15 dilution technique may be suitable for estimating dinitrogen fixation by faba beans in the field using barley or canola as reference crop.

The objectives of this study were to measure N-15 allocation among above and below ground components of crops grown in N-15 labelled soil; and to examine two potential reference crops for field measurement of dinitrogen fixation by faba bean using N-15 dilution techniques; and to compare measurements of dinitrogen fixation calculated using two reference crops and isotope dilution with TDM methods.

3.2. Materials and methods

3.2.1 Description of study site

This study was carried out on the Soil Science plots at Breton (NE-25-47-4W_5), which is 110 km Southwest of Edmonton, Alberta. Dark Gray Luvisols and Gray

Luvisols predominate at the plots (Lindsay *et al* . 1968). The aspect is southwest. Some chemical properties of the soil are presented in Table 2.1.

3.2.2 Plot establishment and N-15 application

As described previously (Chapter 2) open-ended steel cylinders (20cm diameter, 30cm H) were used in this study. Faba bean (*Vicia faba* (L.) minor. cv. Ackerperle), canola (*Brassica napus* (L.) cv. Westar) and barley (*Hordeum vulgare* (L.) cv. Empress) were the crops. Treatments were faba bean, canola, barley and summer fallow, replicated four times. Crop seeding and plot establishment were described in Chapter 2. Canola was reseeded due to a snow storm in late May. Cylinders were pushed into the soils on June 15, 1987 after appearance of seedlings and followed by application of N-15 enriched urea on June 19. N-15 solutions were made from 99.5% N-15 urea (MSD Isotopes, Division of Merck Frosst Canada Inc., Montreal) and analytical grade urea to make two solutions with 22.017 and 22.049 atom% N-15 (53.763 mg N ml^{-1} and 32.264 mg N ml^{-1}). The applied N at each time corresponded to 4.99, 4.99,4.86 and 4.99 kg ha^{-1} for the 1st, 2nd, 3rd and 4th applications, respectively. Therefore, cylinders which remained till the last sampling had received 19.8 kg N ha^{-1}. During application of N-15 labelled urea solution, aliqouts were applied to several spots in each cylinder with a syringe and needle to a depth of 2cm. Caution was taken not to allow solution to contact any above ground living part of the plants. No efforts were made to promote or prevent contact with underground roots. Calculations of N-15 enriched urea needed were based on an estimated mineralization rate of 20 kg N ha^{-1} yr^{-1} from indigenous soil organic matter based on experience at that site over many years.

A single stock solution 22.049 atom % abundance N-15 was used on the 1st, 3rd and last application dates, while a second one with N-15 22.017 % (32.264mg N ml^{-1}) was used for the 2nd application only.

3.2.3 Sampling schedules and methods used

Samples were collected four times during the growing season: July 8, July 24, August 19 and September 1, 1987 corresponding to 63, 77, 103 and 115 days after seeding barley and faba bean, but 38, 52, 78 and 90 days for canola. Plants were dried and seperated, and soil in the cylinders was removed for analysis and root washing as described in Chapter 2.

3.2.4 Sample preparation and analytical methods

Samples were analyzed for total N and N-15 abundance on an ANA-SIRA Mass spectrometer (VG ISOGAS Ltd., Middlewich, Cheshire, England), which comprises an automated Dumas system (Carlo Erba) for total N and a flow through system of the nitrogen gas so generated for isotope ratio analysis using a triple collector system.

3.2.5 Calculations

N-15 abundance of air was taken as 0.3663% as reported by Junk and Svec (1958).

$$\text{N-15 excess \%} = \text{N-15 abundance in sample\%} - 0.3663\% \quad (1)$$

$$\text{N-15 excess \% in crop} = \Sigma(\text{N-15 excess \% x N in one fraction}) \times 100 \,/\, \Sigma(\text{N in each fractions}). \quad (2)$$

$$\text{N fixed \%} = \frac{\text{N-15\% excess in reference crop} - \text{N-15\%excess in legume}}{\text{N-15\% excess in reference crop}} \quad (3)$$

$$\text{N fixed (kg N ha}^{-1}\text{)} = \text{total N in legume tops x N fixed \%} \quad (4)$$

3.2.6 Statistical analysis

Data collected from this study were subject to analysis of variance for treatment, sampling date, and depth effects on the three crops using BMDP statistical software (Dixon 1983). Newman-Student-Keuls multiple comparisons were conducted where appropriate and when the F-test was significant (Sokal and Rohlf 1981) .

3.3 Results

3.3.1 Above ground components

Crop species, sampling dates, and interactions of crop and sampling effects on N-15 excess were all significant ($p < 0.01$). N-15 excess in faba bean tops differed significantly from canola and barley at all four samplings ($p < 0.01$). N-15 excess increased from 0.291-0.772%, 1.994-4.236% and 1.854-4.082% in faba bean, canola and barley tops, respectively, corresponding to N-15 applied. N-15 excess in faba bean tops did not change significantly over time, but that in canola tops significantly increased between the 1st and the 2nd, and the 3rd and 4th sampling; and that in barley tops increased till the 3rd sampling then decreased on the last sampling (Fig. 3.1). N-15 excess (%) in

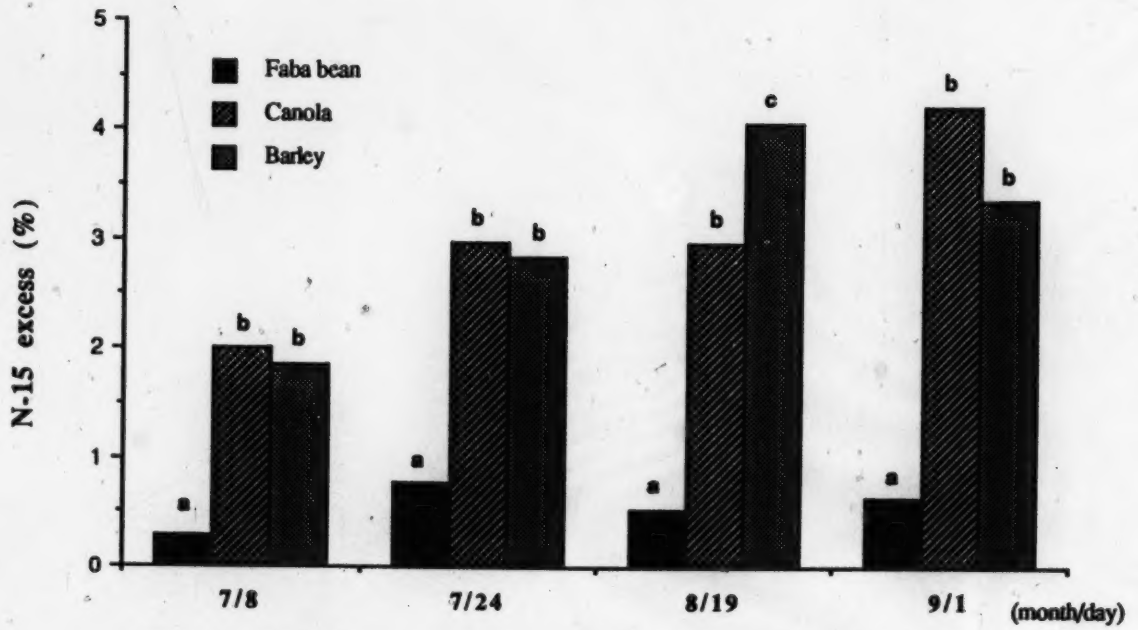

Fig. 3.1 Variations of percentage N-15 excess in crop tops

a-c means followed by the same letters do not differ significantly on one date ($p < 0.01$)

barley and canola tops were not different on the 1st, 2nd and 4th sampling dates; on the 3rd sampling date, barley was statistically and significantly higher than canola. On all sampling dates, faba bean tops had a significantly lower N-15 excess (%) than did either barley or canola (Fig. 3.1).

Normally, reference crops should have the same atom% excess N-15 to be considered equally useful for calculations of dinitrogen fixation. There is a need to comment on whether barley and canola had the same enrichments. Faba bean leaves and stems had almost identical N-15 excess except on the fourth sampling where stems had 0.762% excess N-15 and leaves 0.682% excess (Fig. 3.2a). Between the 2nd and the 3rd samplings, there was a decrease of N-15 excess by 29-30% in leaves and stems following onset of flowering and rapid growth and N accumulation as presented in Chapter 2. During this period (2nd - 3rd sampling), N-15 excess in seeds, a further reduction below that in leaves, stems or husks, but the latter three had identical N-15 excess. As the crop developed, differences in N-15 excess increased, with N-15 excess following: stems > leaves > husks > seeds. Apparently, the assimilated highly enriched soil N was transported more into the vegetative organs, such as leaves and stems; whereas seeds formed a stronger sink for symbiotically fixed N, which is of lower N-15 enrichmen during this period after faba bean was in bloom. During this period between the 2nd and 3rd sampling, N-15 excess in faba bean components dropped, particularly in seeds, indicating a peak in symbiotic dinitrogen fixation.

N-15 excess in canola tops differed from that in faba beans and increased over sampling dates in the growing season. N-15 excess in various components of canola were within 9.0% of each other on the 3rd sampling and 5.9% on the last sampling (Fig. 3.2b). N-15 excess in leaves and stems of canola was the same as that for barley leaves on the 1st sampling, and there was an equal allocation of N from soil and fertilizer to the two components. On the 2nd sampling, leaves and stems had been enriched by 55 and 40% compared with the 1st sampling. Since more N had accumulated in leaves than in stems (Chapter 2), inorganic N was transported to leaves more than to stems and roots, thereby becoming slightly more enriched by fertilizer N. On the last sampling when canola and barley were fully mature, N-15 excess followed a decreasing sequence: seeds > shells > stems. Leaves had disappeared by the last sampling.

N distribution in various components of barley tops followed a decreasing order as: seeds > husks > sheaths > stems > live-leaves > dead-lives in the growing season (Fig. 3.2c). Between the first two samplings, N-15 excess increased by 30% in various

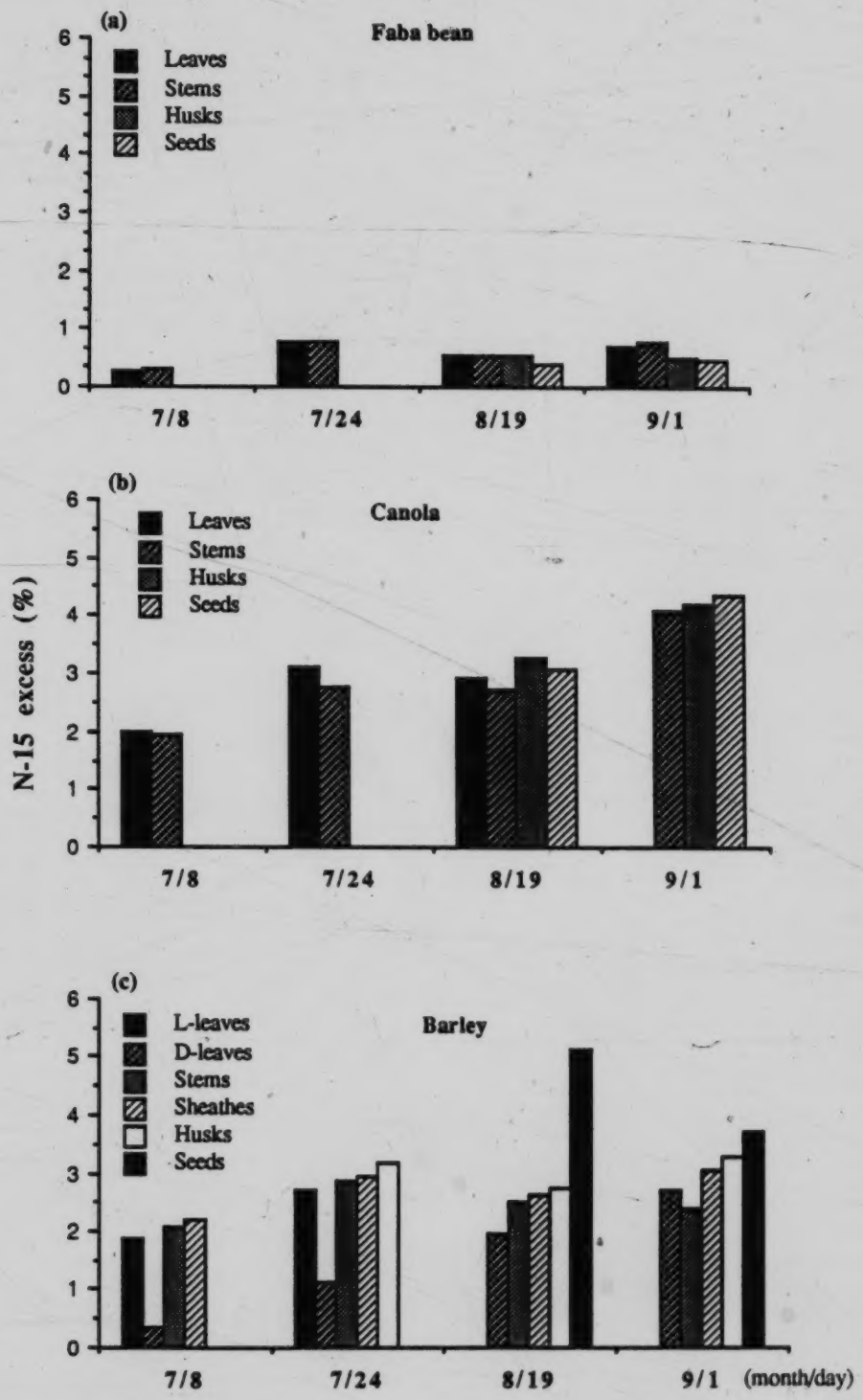

Fig. 3.2 N-15 excess in various components of crop tops

components and also followed a decreasing order as above. N-15 excess of dead-leaves was 81-83% lower than that in live-leaves, stems and sheaths on the 1st sampling; 59-62% on the 2nd sampling. During late development, N-15 excess increased markedly in seeds on the 3rd sampling and decreased but was still higher than in other components on the last sampling. The high enrichment of N-15 in seeds on the 3rd sampling corresponded to the grain-filling stage and the disappearance of live-leaves.

By fractionating the plants into several physically important parts, each with its own ontogeny, it has been possible to examine to what extent the source of N to the crops has been temporally uniformly distributed between fertilizer, soil and atmosphere, and how that N has been allocated. These data suggested that in spite of the small amount of N applied, considerable temporal variability existed in N source; but it did not affect N-15 excess in faba bean, and was generally equally expressed in canola and barley. Immature barley seeds on the 3rd sampling were the most sensitive to recently added fertilizer N-15.

3.3.2 Below ground components

N-15 excess in the 0-27cm depth of nonlegumes (canola and barley) roots increased consistently after N-15 application over the four samplings conducted (Fig. 3.3). N-15 excess of faba bean roots was lower than that of the nonlegumes and did not increase consistently in response to continuing N-15 application . It was 42-53, 25-39, 47-61 and 47-55% lower than that in barley or canola on the four samplings conducted, respectively. N-15 excess was 18-37% higher in canola roots than in barley. The decrease of N-15 enrichment in faba bean roots on the 3rd sampling corresponded to depletion of N-15 in faba bean tops and the rapid increase of crop tops at the 3rd sampling. Root dry matter of canola and barley did not change significantly over the four samplings (Chapter 2); yet N-15 excess in the two nonlegume roots increased continuously (Fig. 3.3). N-15 % excess was consistently and slightly lower in faba bean tops than roots, but it was 2 to 4 fold higher in canola and barley tops than in their roots.

Crop species, sampling dates, depth, and interactions of crop and depth effects on N-15 excess were significant ($p < 0.01$). Interations of sampling and depth effects were significant on N-15 excess ($p < 0.05$). Interactions of crop and sampling, and of crop, sampling, and depth effects were not significant on N-15 excess ($p > 0.1$). Highest N-15 excess was found in canola at 0-10cm depth followed by barley and faba bean at the same depth. Generally, there were no significant differences in N-15 excess between 10-20 and 20-27cm, and between species of crops at these two depths (Fig. 3.4). N-15 enrichment

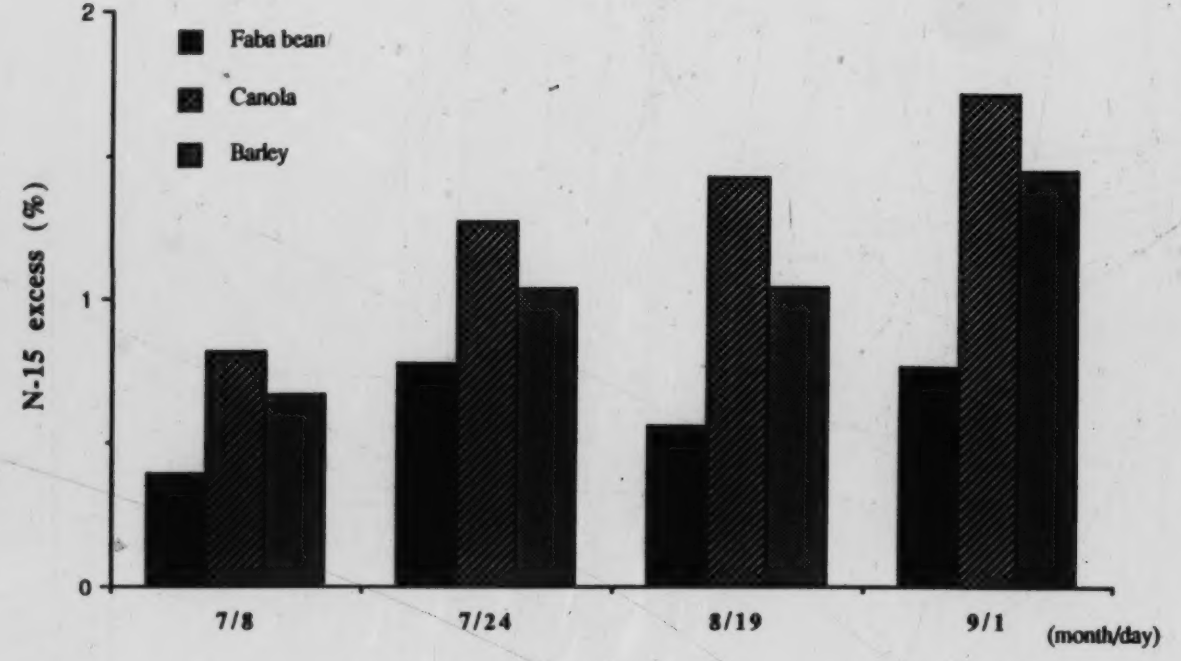

Fig. 3.3 Percentage N-15 excess in crop roots (0-27cm)

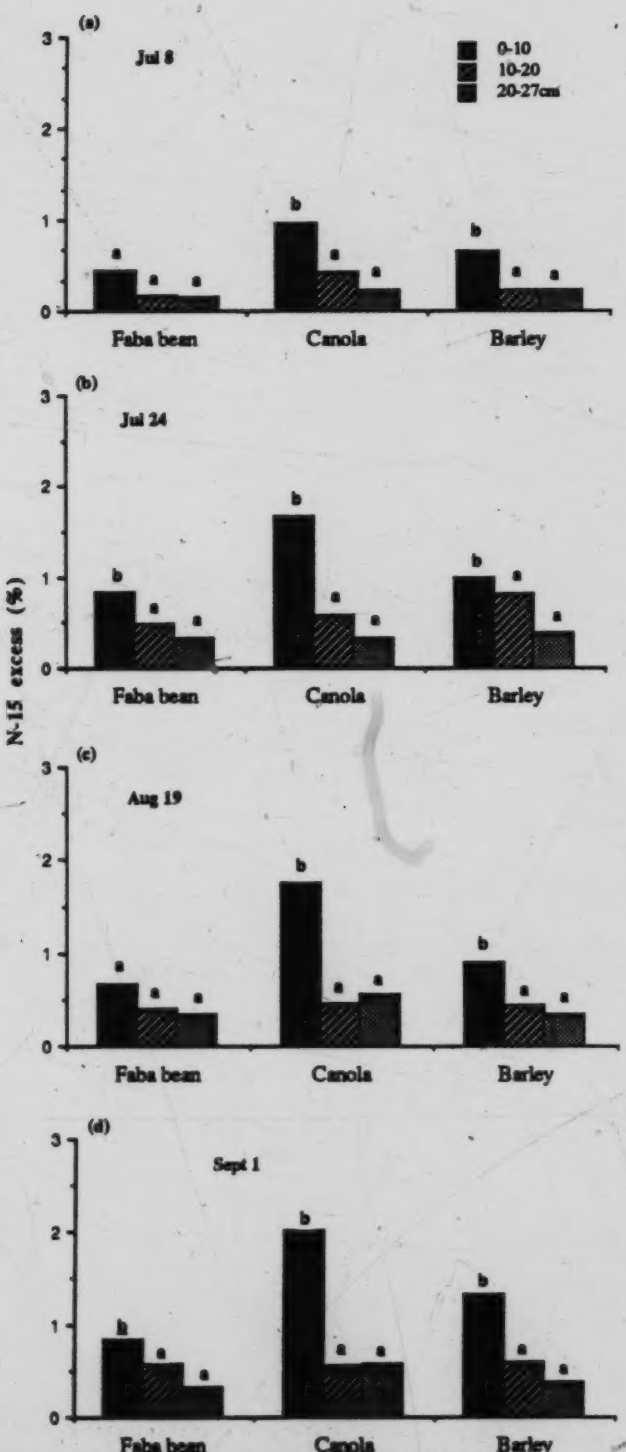

Fig. 3.4 N-15 excess in crop roots over one growing season

a-b means followed by the same letters do not differ significantly on one date ($p < 0.01$)

was higher in canola roots than in barley on all samplings. Roots for these three crops were concentrated in 0-10cm with a denser, compacted layer of soil beyond. Bulk density of the soil was 1.1, 1.3 and 1.6 Mg m^{-3} for 0-10, 10-20 and 20+ cm (Dinwoodie 1988). Lower roots consequently have poor accessibility to N-15 in the lower depth due to the restrictive physical condition for growth and development as well as the limited movement of applied N-15. Root nodules of faba bean were mostly distributed within 0-10cm, a few were observed between 10-27cm. The large nodules were near tap roots and within 10cm depth.

3.3.3 Quantity of N fixed in faba bean

N-15 excess in the legume crop (faba bean) differed significantly from that in either canola or barley. Only crop tops were considered to estimate dinitrogen fixation in faba bean for comparing with data reported. Using either canola or barley as a reference crop with the N-15 dilution technique yielded similar results. (Table 3.1). The percentage of N fixed in faba bean tops on September 1 calculated using canola or barley as a reference crop varied between 70-76%, which corresponded to 183-199 kg N ha^{-1} yr^{-1}. There was an increase of dinitrogen fixed in faba bean between the 2nd and 3rd samplings, and it was 7 and 15% increase with canola and barley as reference crops. This increase corresponded to an absolute increase in N of 104 and 122 kg N . The variation in quantity of dinitrogen fixed between reference crops was due to the cascading of N-15 excess in canola and barley between the 3rd and 4th sampling. Dinitrogen fixed in faba bean tops was 104-122

Table 3.1 Dinitrogen fixation in faba bean tops (N-15 dilution method)

Ref. crop	7/8	7/24	8/19	9/1
		(month/day)		
		(%)		
Canola	72	66	73	76
Barley	70	65	80	70
		(kg N ha^{-1} $year^{-1}$)		
Canola	53	65	169	199
Barley	52	64	186	183

kg N ha^{-1} yr^{-1} between the 2nd and the 3rd samplings. The rate of dinitrogen fixation in faba bean was calculated based on the quantitative data from N-15 dilution. It reached 4.0-4.7 kg N ha^{-1} day^{-1} using canola or barley as a reference crop (between July 24 and August 19). It was 0.75 kg N ha^{-1} day^{-1} between the 1st and 2nd sampling, and less than 2.3 kg N ha^{-1} day^{-1} between the 3rd and last sampling (Table 3.2). A peak of dinitrogen fixation in faba bean after flowering (2nd sampling) was evident.

Table 3.2 Dinitrogen fixation rate in faba bean tops (N-15 dilution method)

Ref. crop	7/8	7/24	8/19	9/1
		(month/day)		
		(kg N ha^{-1} day^{-1})		
Canola	---	0.75	4.0	2.3
Barley	---	0.75	4.7	-0.2

Other methods are also available for quantification of N fixed in faba bean. Among these the total difference method is conventionally used based on crop dry matter production and Kjeldahl analysis of N. In this study, TDM is comparable to the N-15 dilution method when crop tops were considered in the calculation; but the results from TDM were slightly higher than N-15 dilution over all samplings. The variation between the N-15 dilution and TDM is within 10% (< 20 kg N ha^{-1} yr^{-1}). The N fixed in faba bean tops was 215 kg N ha^{-1} yr^{-1} using TDM, and the percentage of N derived from N fixation was 82-86% (Table 3.3). This agreement is much better than that between N-15 and acetylene reduction as reported (Smith and Hume 1987). The more widely applicable Kjeldahl method would permit dinitrogen fixation by faba beans to be quantified on such soils without reliance on N-15 method.

Crop roots are normally neglected in dinitrogen fixation studies, but roots were extracted from soil by a root-washing technique in this study. N in the roots derived from the atmosphere accounted for 19-49% of the N accumulated in roots, which was 18-22 kg N ha^{-1} yr^{-1} using N-15 dilution with canola and barley as reference crop (Table 3.4). Therefore, actual dinitrogen fixation in faba bean may be as high as 201-221 kg N ha^{-1} $year^{-1}$.

Table 3.3 Dinitrogen fixation in faba bean tops (total difference method)

Ref. crop	7/8	7/24	8/19	9/1
		(month/day)		
		(%)		
Canola	69	76	84	86
Barley	70	67	83	82
		(kg N ha^{-1} $year^{-1}$)		
Canola	51	75	195	225
Barley	52	66	192	215

Table 3.4 Dinitrogen fixation N in faba bean roots (N-15 dilution method)

Ref. crop	7/8	7/24	8/19	9/1
		(month/day)		
		(%)		
Canola	36	30	49	46
Barley	27	19	34	38
		(kg N ha^{-1} $year^{-1}$)		
Canola	9.0	8.0	20	22
Barley	6.7	5.1	14	18

3.4 Discussion

3.4.1 Above ground components

Theoretically, the N-15 dilution technique is valid only under conditions where: 1). both legume and nonlegume assimilate soil N and N-15 enriched fertilizer at the same ratio, but not necessarily the same quantity; and 2) no fixed N-15 is transferred from legume to nonlegume in inter-cropping systems, particularly under pasture. The second condition was eliminated in this study by setting treatment plots for legume and nonlegume separately, and also by applying highly enriched N-15 material to mask the background

variation among plots cropped to legume and nonlegume. However, the first condition was evaluated indirectly by comparing N-15 assimilation between the legume and two nonlegumes.

The reference crop constitutes the principle source of error in the N-15 technique for determining N fixed in field studies. There is no general agreement on the best nonfixing reference plant though different alternatives are available. Rennie (1982) reported N fixation in soybean cultivars was overestimated when non-nodulating isolines were used, and the uninoculated and ineffectively inoculated treatments were the best controls. Of three non-legumes tested, barley was considered to be useful as a reference crop, but rapeseed and sudangrass were not. Wagner and Zapata (1982), however, suggested that non-nodulating soybean and sudangrass were as good as uninoculated soybean as reference crops for soybean. Barley was considered to be a very satisfactory reference crop for *V. faba* (Wagner and Zapata 1982), but Chalk *et al.* (1983) suggested that the nonfixing reference crop could be replaced by N-15 mineralized from N-15 pre-enriched soil in which significant N-15 excess of inorganic N derives from soil organic matter N.

In this study, canola and barley were chosen as candidate reference crop. N-15 excess in the legume (faba bean) differed from nonlegumes (canola and barley) significantly ($p < 0.01$), but there was no statistically significant difference in N-15 excess between canola and barley except on the 3rd sampling date. Deviation of N-15 excess in canola and barley tops in the later growing season will cause the quantity of dinitrogen fixed in faba bean calculated from N-15 dilution to vary. Variation caused by nonlegumes is only 6% in terms of percentage dinitrogen fixed in faba beans on the last sampling. These results provided a reliable, valid basis for further quantification of N fixed in faba bean using either canola or barley as a reference crop. On the 3rd sampling, barley had 27% higher N-15 excess than canola, but canola N-15 excess was 21% higher than in barley on the last sampling. Therefore, it is reasonable to postulate N fixed in faba beans can be estimated equally well with either canola or barley as a reference crop using N-15 isotope dilution and cylinders to contain N-15 in soils with a restricted rooting zone.

A significant dry matter increase was observed between 2nd and 3rd sampling which coincided with flowering (Chapter 2). The 32% depletion of N-15 excess in faba bean during this interval further indicates an increased rate of dinitrogen fixation. Similar results were reported by Richards and Soper (1979).

Fixed N was preferentially transported to reproductive organs after entering the stage of generative growth. It should be noticed that N-15 excess of faba bean seeds on the 3rd sampling was 25% lower than in leaves, stems and shells, which are pre-seed formed organs. The interpretation is that N, which is mostly of atmospheric origin, was transported into seeds in preference over other components. With further development, N-15 excess in leaves, stems and seeds increased by 25, 39 and 15% but decreased by 6% in shells. Because total N accumulated in seeds was much higher than in other components (Chapter 2), a small increase of N-15 excess could mean a large amount of N translocated. Nutrient allocation within the plant is governed by development stage of a crop; the shift of growth from vegetative to reproductive stage was accompanied by shifts in N-15 excess among parts of the bean plants.

N is translocated at different rates to different plant parts, depending on the state of maturity. The N pool absorbed by plants at different periods is not of the same N-15 enrichment, however. Since different amounts of N of similar N-15 enrichments are translocated to diverse parts at various physiological stages, it is not surprising to find that individual plants have different $^{15}N/^{14}N$ ratios as shown in Fig. 3.2. For example, during barley growth, N-15 excess in dead-leaves was much lower, by 6-7 fold, because N can be translocated to new developing components of crop. These differences in isotopic composition within different plant parts constitute an important potential source of error in N fixation studies. A proper sampling for N-15 analysis is difficult when plant parts of different densities such as seeds, pods, stems, and leaves are in the same sample. This can be overcome by fractionating plants into recognizable plant parts, each of which is physically similar and is homogenous in N-15 composition.

3.4.2 Below ground components

Zapata et al (1987) stated in their study that N contribution from roots was small and did not take it into consideration. In my study, the roots of faba beans had a higher N-15 excess than their tops by 3-19%; conversely, canola and barley roots had lower N-15 excess than the tops (Fig. 3.3). This discrepancy in N-15 excess patterns over the four samplings between crop tops and roots indicates biological fractionation may not be neglegible in N-15 applied research. Kohl *et al.* (1982) found that the nodules of soybean are more enriched in N-15 than the rest of the plant and the enrichment increased with time. They suggested a process, other than dinitrogen fixation itself, which is associated with dinitrogen fixing activity, caused elevation of N-15 abundance in soybean nodules. The mechanism involves isotopic fractionation associated with synthesis of compounds rich in

N-14 which are then exported from the nodule leaving behind N-15 enriched metabolites for the synthesis of nodule tissue. Ruschel and Sauito (1986) reported soybean roots did not contain N from fixation and an assumption was made that symbiotically fixed N was primarily distributed to tops of the nodulated plants. But, fixed N was found in roots when N-15 labelled nitrate was applied. Plant roots may depend on N assimilated from soil while above ground faba bean growth uses synthesized N transported in forms of glutamine and asparagine from nodules. The combination of elevated N-15 excess in faba bean root and lowered N-15 excess in canola and barley roots (relative to tops) yields less than 20% of faba bean root N but over 70% of above ground N derived from the atmosphere.

3.4.3 Quantity of N fixed in faba bean

With total difference method (TDM), Richards and Soper (1982) estimated N fixation accounted for 63-71% of the N in faba bean shoots, which was 51-111 kg N ha^{-1} yr^{-1} in Manitoba. Wagner and Zapata (1982) reported between 71-84% corresponding to 125-144 kg N ha^{-1} yr^{-1} using the A-value method In a recent report, Zapata *et al.* (1987) concluded that 79% N in faba bean was derived from fixation (165 kg N ha^{-1} yr^{-1}) using the N-15 of the dilution technique. A larger amount of N appears to have been fixed in faba bean at Breton. By September 1, when faba beans were still not fully mature, the percentage of N derived from N fixation varied between 70 and 76% corresponding to 183-199 kg N ha^{-1} yr^{-1} with either canola or barley as reference crops at Breton (Table 3.1). Faba bean root N derived from fixation amounted to 18-22 kg N ha^{-1} yr^{-1}; therefore, the total N fixed in faba bean tops and roots was 201-221 kg N ha^{-1} yr^{-1} at the Breton plots.

In conclusion, using canola or barley as a reference crop for N-15 dilution method, yielded results which varied by about 5%. Wagner and Zapata (1982) similarly concluded that barley, sudangrass or oilradish were equally effective as a reference crop. The total difference method yielded results which were about 10% higher than the isotope dilution method. At Breton on a Gray Luvisol, the total difference method appears to be a comparable one to estimate dinitrogen fixation because the N-15 method is limited by expense and time. A peak of N fixation was observed after faba bean flowering (between the 2nd and the 3rd samplings), and the rate of N fixation reached 4.0-4.7 kg N ha^{-1} day^{-1} using canola or barley as reference crops. Before or after this period, dinitrogen fixation rate was much lower. The total quantity of N fixed by faba bean over the season was estimated to be between 201-221 kg ha^{-1} when roots are included; with 9-10% of the total in roots within the top 27cm of soil.

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CHAPTER 4. MICROBIAL BIOMASS C AND N DYNAMICS, AND N-15 INCORPORATION INTO MICROBIAL BIOMASS UNDER FABA BEAN, CANOLA, BARLEY AND SUMMER FALLOW ON A GRAY LUVISOL[3]

4.1 Introduction

Soil microbial biomass is a biologically active fraction of the soil organic matter and is the driving agent for nutrient cycling. It governs the rate of turnover and mineralization-immobilization of organic substrates in soil (Jansson 1958; Paul and Juma 1981; Paul and van Veen 1978). Despite the small quantities of biomass (1-2% soil organic C and 3-5% of soil N), its role in the transformation of organic matter as a sink and a source of nutrients is crucial. Dynamics of microbial biomass in soil have been studied with ^{14}C and ^{15}N tracer techniques (Paul and van Veen 1978; Paul and Juma 1981); and mathematical models developed to simulate microbial mineralization and immobilization of nitrogen in culture conditions (Van De Werf and Verstraete 1987a; 1987b; 1987c), laboratory incubations (Paul and Juma 1981) and a grassland system (McGill *et al.* 1981) based on experimental data to varying degrees. Models have provided insight about control mechanisms, which are hard to quantify due to methodological problems (van Veen *et al.* 1981).

Soil microorganisms have long been considered as a labile pool of C, N, P and S and consequently the turnover of these elements through the activities of microorganisms is very important for nutrient flow (Jenkinson and Ladd 1981). Microbial biomass in agricultural soil is affected by crop rotation (McGill *et al.* 1986), tillage (Carter and Rennie 1982; Carter and Rennie 1984; Lynch and Panting 1980a), soil texture (van Veen *et al.* 1987) and alternating moist and dry conditions (Bottner 1985). Generally, exogenous substrates are in very low concentrations so that growth and activity of the biomass is restricted (Tateno 1988), and the small amounts of substrate available are used for maintenance. Hence, understanding the relationships between microbial biomass in soil and agricultural management practices will aid in further understanding and managing nutrient flow on a terrestrial scale (McGill and Myers 1987). Rates of turnover of nutrients through the soil biomass and mechanisms controlling them are still not well understood despite the need for such quantitative descriptions to form the basis of nutrient management strategies for specific soils.

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[3]A version of this chapter will be submitted for publication by Gu, J.-D. and W.B. McGill.

Microbial biomass in soil is closely associated with plant growth (Lynch and Panting 1980a; 1980b) and much of the biomass in cropped soil is in the rhizosphere (Barber and Lynch 1977). Cereal roots release organic substrates into the soil (Barber and Martin 1976); the extent of such release is affected by rhizosphere conditions and crop species. Crops alter the soil environment in various ways and therefore biomass dynamics may be expected to vary in soils supporting diverse plant species. Further, N fixed by legumes may become available, during the growing season, for microbial biomass in the rhizosphere of legumes.

The specific objectives were: i) to estimate microbial biomass C and dynamics of the flush of N from biomass in soils cropped to faba bean, canola, barley and a noncropped summer fallow; and ii) to trace N-15 incorporation into the microbial biomass fraction using N-15 labelled urea under cropping (faba bean, canola and barley) and noncropping (summer fallow) conditions.

4.2 Materials and methods

4.2.1 Soil Samples Used

This study was conducted at the University of Alberta Soil Science Plots at Breton (NE-25-47-4W_5), which is 110 km southwest of Edmonton, Canada. Dark Gray Luvisols together with Gray Luvisols predominate in the study area (Lindsay *et al.* 1968). The experimental design comprised four-treatments (faba bean, canola, barley and summer fallow) replicated four times. Open-ended steel cylinders (30cm long and 20cm diameter) were used to contain N-15 labelled microplots within each macroplot (Chapter 2 and 3). Four cylinders were placed in each replicate plot and the cylinder was designed with two 0.5cm diameter holes on opposite sides of the wall 1cm below the upper end so that the holes were at ground level after the cylinders were pushed well into the soil and could function to discharge flooded water following heavy rain falls.

N-15 labelled urea was added four times: June 19, July 8, July 24 and August 19 in 1987 to the surface 2cm soil depth within each cylinder. Soil samples were taken 4 times during the growing season: July 8, July 24, August 19 and September 1, 1987 prior to adding N-15 enriched solution. Therefore, N-15 was added to the remaining cylinders only after each respective sampling. A syringe was used to inject solution N at each time corresponding to 4.99, 4.99, 4.86 and 4.99 kg N ha^{-1} to 2cm depth on the four applications. N-15 solutions were made from 99.5% N-15 urea (MSD Isotopes, Division

of Merck Frosst Canada Inc., Montreal) and analytical grade urea to make two solutions with 22.017% N-15 and 22.049 % N-15(53.763 mg N ml^{-1} and 32.264 mg N ml^{-1}). Therefore, cylinders which had remained till the last sampling had received N corresponding to 19.8 kg ha^{-1}. Further descriptions of the experimental set up are available (in Chapters 2 and 3). Soil samples were taken as described earlier and stored at 4°C prior to analyses for microbial biomass C and N about four months after the first sampling. These soil samples were passed through a 10-mesh sieve and visible roots removed. Analysis for soil microbial biomass C and N used composite samples of four replicates from each treatment. A set of soil samples was taken at the last sampling date (September 1, 1987), and some chemical properties of the soil were analyzed. Resuts are presented in Talbe 2.1.

4.2.2 Biomass C and N Determination

Soils were equilibrated over night at 22°C before fumigation. The chloroform fumigation incubation technique (CFIT) of Jenkinson and Powlson (1976a; 1976b) was used in this study to measure C and N mineralized from microbial biomass. Soils were extracted with 2M KCl on the day of fumigation and also after a 10-day period of incubation at 22°C for both fumigated and nonfumigated soils.

The equivalent of 25g dry weight soil was taken from each 0-10cm sample. Two samples were fumigated and another two were left as controls. CO_2 evolved from each sample was trapped in 20ml 0.25M NaOH in a 2L Kerr jar and titrated with 0.5M HCl on a Memotitrator (Mettler DL40RC, Switzerland) after adding 5ml 15% $BaCl_2$ to precipitate trapped CO_2 as $BaCO_3$. The quantity of microbial biomass C was calculated using Kc = 0.411 as follows:

$$\text{Biomass-C} = (\text{CO}_2\text{-C evolved from fumigated soil} - \text{CO}_2\text{-C evolued from nonfumigated soil}) / 0.411 \quad (1)$$

Mineral N in the 2M KCl extract of soils before fumigation, and of fumigated and nonfumigated soils after incubation, was determined by steam-distillation with MgO (0.5g) for NH_4^+-N followed by Devarda's alloy (0.2g) for (NO_3^-+NO_2^-)-N (Bremner and Mulvaney 1982) after cooling to near room temperature. For those samples containing less than 100µg N, a modified diffusion method was applied (procedures are described in the following section). Samples were run in duplicate. During steam distillation, the recoveries were 98% for ammonia and 93% for nitrate respectively in a step-wise sequence

as for samples. Total mineral N was determined with the same standard solution, and a recovery of 97% was obtained. Biomass N was not calculated due to discrepancies in reported values for Kn (Voroney and Paul 1984); instead, the flush of N was used throughout in this paper. Flush of N was calculated as:

$$Fn = F(NH_4^+)_{10} - F(NH_4^+)_0 \qquad (2)$$

NH_4^+ from both steps of distillation was trapped in 5ml 2% boric acid and then titrated with dilute H_2SO_4 on a Memotitrator (Mettler DL40RC, Switzerland). All samples were acidified with 2 drop 0.5M H_2SO_4 after titration to a pH approximately 3.4, and dried on an 80°C sandbath. Salts were redissolved and transferred to culture tubes and oven-dried at 65°C for N-15 analysis on the ANA-SIRA Mass Spectrometer (VG ISOGAS Ltd., Middlewich, Cheshire, England).

4.2.3 N-15 diffusion technique

For samples that contained less than 100μg N/sample during acid titration an N-15 diffusion technique was applied (P.D. Brooks, personal communication). This is a modified procedure from Turner and Bergersen (1980). A solution (20-30ml) was placed in a 140-ml plastic specimen container, and a 6mm(diameter) disk , cut from Whatman GF/D glass fiber filter paper with a paper punch, was put on a #22 solid PVC coated wire (type MW-U, MIL-W-76B) in the middle portion of this wire. Ten μl of 2.5M $KHSO_4$ was pipetted onto the disk and a small scoop of MgO (0.2g) was added to solution in the container. Then the wire with the disk on it was placed in the plastic container with both sides of the wire extended to the wall of the container, so that the disk was suspended above the solution. The container was capped tightly and swirled to let MgO become well mixed with solution. The containers were left at room temperature (22°C) for 6 days for diffusion of NH_3 into the KHSO4 moistened disk. The container were opened and the wire removed. The wire was carefully tilt so that lightly striking it with the thumb at the lower end moved the disk near to that end of the wire. The wire was bent from the middle to 90-120°. The wire can be pushed into styrofoam by twisting while the disk remains on the horizontal portion of the wire. Wire and disks on styrofoam were transfered to a desiccator with a 500ml beaker containing 400ml concentrated H_2SO_4 and sealed. Four days later, N-15 analyses were carried out on these disks using the ANA-SIRA Mass Spectrometer (VG ISOGAS Ltd., Middlewich, Cheshire, England).

4.2.4 Statistical Analysis

Statistical analysis was conducted for ANOVA (P3V), multiregression (P2R) and principal component analyses (P2R) on NH_4^+, in soils before fumigation, and fumigated and nonfumigated soils after 10 days incubation using BMDP statistical software (Dixon 1983). Three crops together with summer fallow were categorized as main treatments whereas four sampling dates were considered as another treatment. Multiple comparison following Student-Newman-Keuls was carried out when a significant difference was found upon treatment effect of ANOVA.

4.3 Results

4.3.1 Soil microbial biomass C and respiration C

The general pattern of respiration in the laboratory and of biomass C was a bell-shaped curve over the four samplings for canola barley and summer fallow, but not for faba bean (Fig. 4.1). Analysis of variance showed that treatment and sampling date effects were significant for microbial biomass C ($p < 0.01$). Microbial biomass C in cropped soils was higher compared with summer fallow except for the last sampling date. It ranged between 131.0 and 429.1 $\mu g\ C\ g^{-1}$ soil in summer fallow, and between 272.1 $\mu g\ C\ g^{-1}$ soil in barley on August 19 and 836.3 $\mu g\ C\ g^{-1}$ soil in canola plots of July 24 (Fig. 4.1). On the 3rd and last sampling, microbial biomass C was significantly higher in the faba bean plot than that in canola, barley or summer fallow plots; there was no significant difference among the latter three. At that date, microbial biomass C in the faba bean plot was 2.4, 1.8, 2.7 fold higher than in canola, barley and summer fallow, respectively.

Microbial biomass C of the faba bean plot did not vary significantly throughout the four samplings in the growing season (range: 575.0-674.0 $\mu g\ C\ g^{-1}$ soil), while that of the barley plot, remaining almost constant during the first two sampling dates, decreased significantly on the 3rd sampling date and increased on the last sampling. In contrast, biomass C under canola increased by more than two fold after the 1st sampling and then decreased to near its original quantity after the 2nd sampling date. The pattern in the summer fallow plot is very similiar to that in canola. At each sampling date, fallow soil had significantly less biomass C than the other treatments, except for the last sampling when it was among a group of three, all with lower biomass C than under faba bean. Over the last two sampling dates, soil under barley and canola had about half as much microbial biomass as under faba bean, with fallow soil averaging the lowest biomass C over all sampling dates.

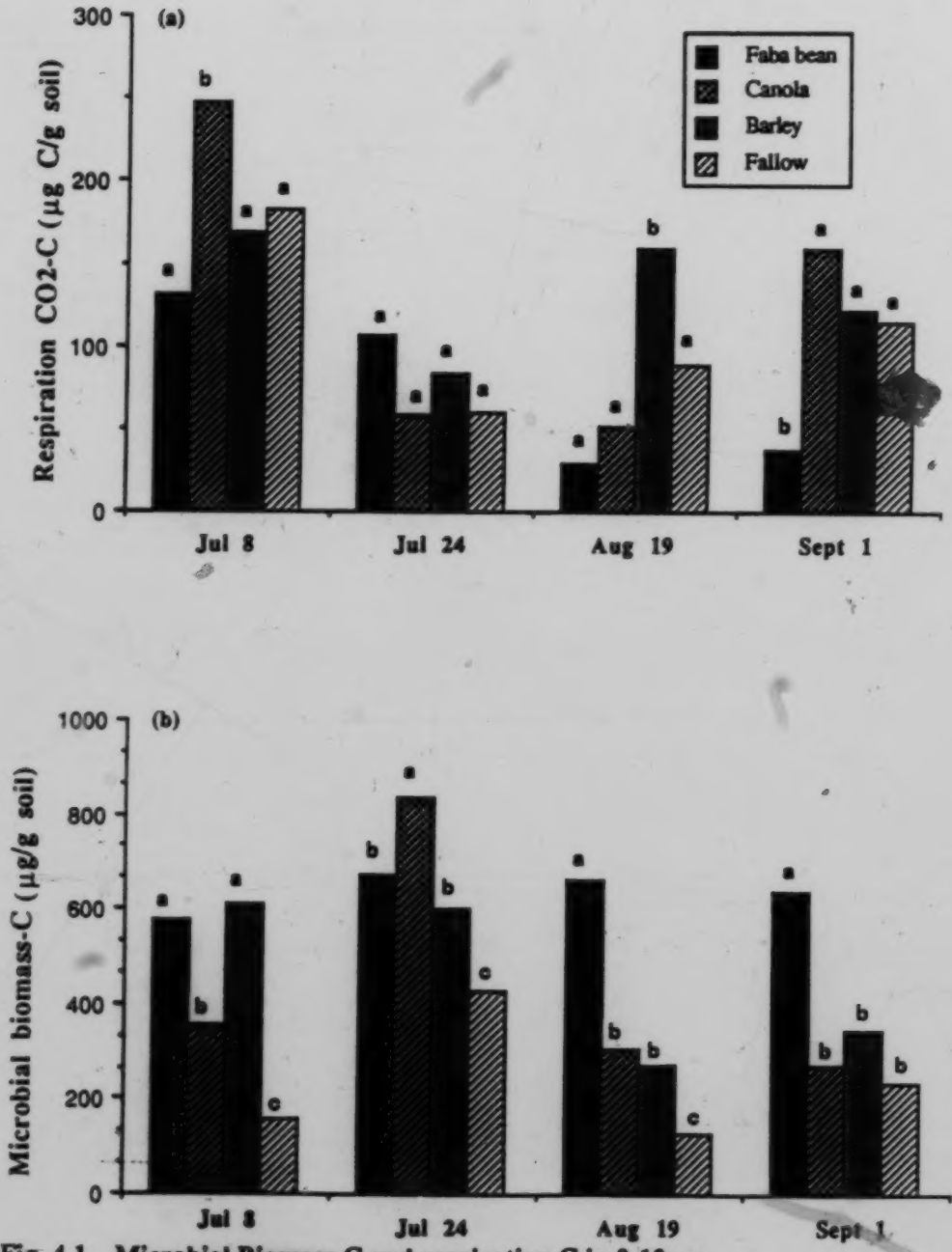

Fig. 4.1 Microbial Biomass C and respiration C in 0-10cm

a-c means followed by the same letters do not differ significantly on one date ($p < 0.05$)

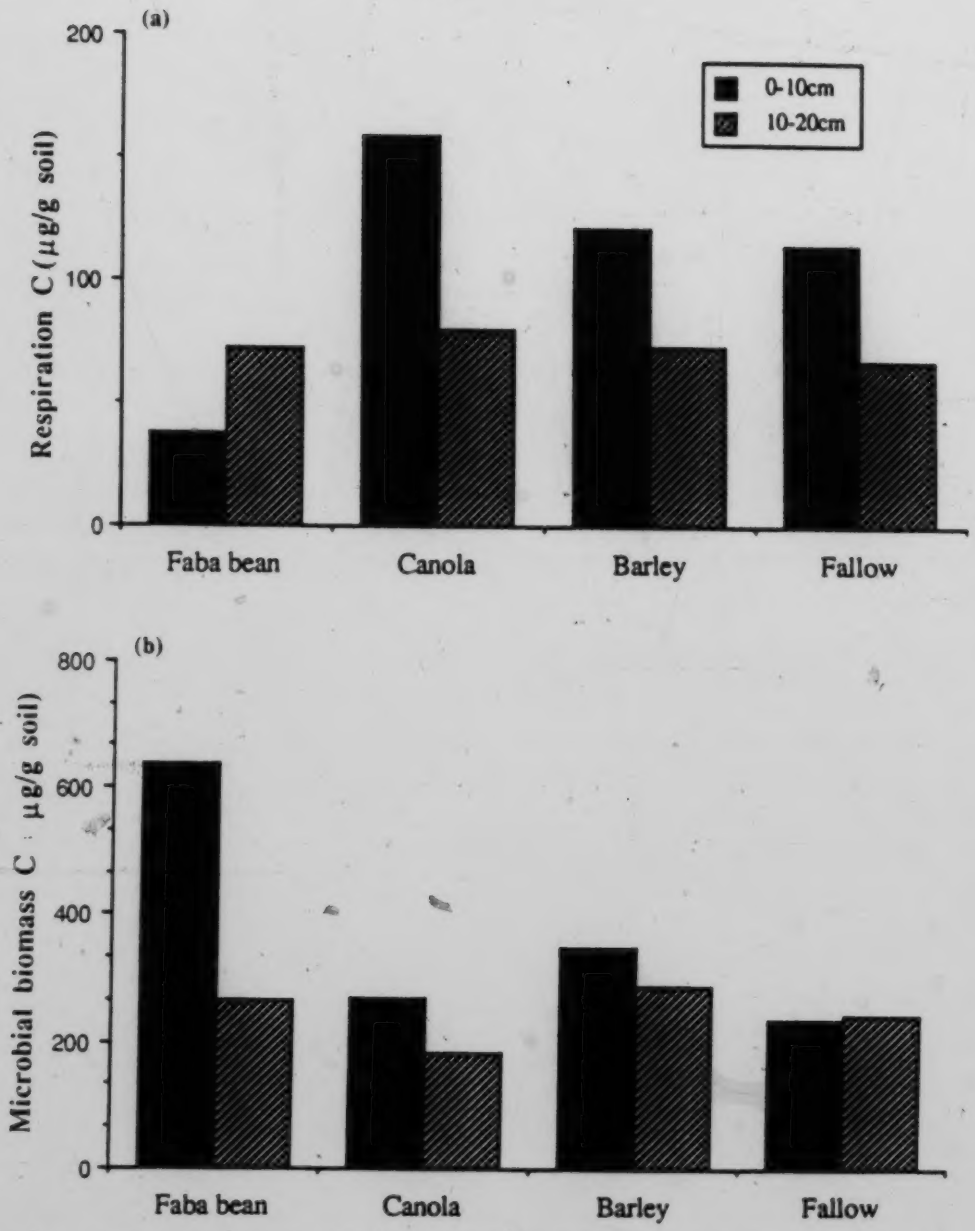

Fig. 4.2 Microbial biomass C and respiration C for 0-10, 10-20cm depth on the last sampling

The CO_2-C evolved during a 10-day incubation of the nonfumigated soils was taken as soil respiration C. Sampling date and treatment effects were significant at $p < 0.01$ and $p < 0.1$, respectively. Their interaction was not significant ($p > 0.1$). Over the four samplings, the trend in respiration both with time and between treatments was almost the reverse of biomass C. Respiration C from faba bean plots was lower than that from the canola, barley or summer fallow plots except for the 2nd sampling and all treatments were low with nosignificant differences among treatments on that date.

Biomass C and respiration C for the last sampling date was lower in the 10-20cm depth compared with 0-10cm except for biomass C under fallow and respiration C under faba beans (Fig. 4.2). The magnitude of biomass C decrease is greater for faba bean followed by canola and barley. Respiration C decreased more dramatically than did biomass C, except under faba beans where respiration was greater at 10-20cm than 0-10cm.

The decreases were 50, 40 and 41% in 10-20cm depth soil cropped to canola, barley, and summer fallow compared to 0-10cm, but increased by 91% in 10-20cm of faba bean.

4.3.2 Flush of N released (Fn)

Fn was calculated in three ways: first, by taking the difference of $NH_4^+ + NO_3^-$ between fumigated and nonfumigated soils at day 10; second, use difference of NH_4^+ in fumigated and nongumigated soils at day 10; and third, use NH_4^+ in fumigated soil at day 10 without subtracting a nonfumigated control. The latter two, which did not consider NO_3^-, correlated very well with each other and the range varied 1% for faba bean, 0% for canola, 6% for barley and 0% for summer fallow. Results obtained without control were higher than with the ones where control was considered. On the other hand, variation was 23% for faba bean, 20% for canola, 23% for barley, and 40% for summer fallow for the ones where both NO_3^- and NH_4^+ were taken into account for estimation of Fn. These results were lower than when calculated by method 2 and 3. The second calculation was used throughout for the data reported.

Significant effects were observed on NH_4^+ for date ($p < 0.05$), fumigation ($p < 0.01$) and their interaction effects ($p < 0.05$); whereas treatment, interactions of treatment x date, fumigation x treatment, treatment x fumigation, and fumigation x treatment x date were not significant ($p > 0.1$). Accumulation of NH_4^+ and a slight depletion of NO_3^-

were evident on cropped and non-cropped soils after fumigation (data not shown). The increase in NH_4^+ caused by chloroform fumigation was approximately 21-93 fold. Compared to the nonfumigated cantrol, the largest amount of NH_4^+ released was at the last sampling date for cropped soils. Variation of Fn was between 16.96 - 24.54 $\mu g\ N\ g^{-1}$ soil for the four samplings of four treatments. The average values of four samplings were 18.30, 20.48, 20.70 and 19.73 $\mu g\ N\ g^{-1}$ soil for faba bean, canola, barley and summer fallow, respectively.

Quantitative N-15 data showed a consistent increase with time of N-15 incorporated into microbial biomass except on July 24 of canola plots. The increase of N-15 incorporation over time for faba bean was 71%, 41% and 91% between samplings; it was 14, 32 and 146% for canola; 61, 74 and 16% for barley; and 11, 85 and 26% for summer fallow (Table 4.1). Microbial biomass C was significantly lower in the summer fallow plots, incorporation of N-15 into microbial biomass exhibited a similar trend.

Table 4.1 Quantities of N-15 incorporated into flush N fraction in 0-10cm depth (ng $^{15}N\ g^{-1}$ soil)

Crop	Jul 8	Jul 24	Aug 19	Sept 1
Faba bean	48.63	83.27	117.51	224.76
Canola	66.64	57.29	75.56	185.80
Barley	45.76	73.57	128.25	149.13
Summer fallow	44.07	48.72	90.30	113.35

Incorporated N-15 ranged between 48.63-224.76, 57.29-185.80, 45.76-149.13 and 44.07-113.35 ng N-15 g^{-1} soil for faba bean, canola, barley and summer fallow plots. Quantities of N-15 in summer fallow were lower than in cropped soils. On the first sampling, N-15 in microbial biomass of summer fallow was equivalent to 91, 66 and 96% that in faba bean, canola and barley plots. It was equivalent to 50, 61 and 76% on the last sampling.

Fn calculated with NH_4^+ in fumigated treatment only was close to the one subtracting NH_4^+ in the control during 10 days incubation, but yielded slightly higher values for all treatments on the four sampling dates. The overestimation was higher by 1-2, 1, 1-6 and 1% in faba bean, canola, barley and summer fallow. Calculation with NH_4^+

and NO_3^- at 10 days for fumigated soil minus the nonfumigated soil yielded lower results than those from NH_4^+ without considering NO_3^-. They are lowered by 17-67, 7-27, 0-23 and 1-31% for faba bean, canola, barley and summer fallow plots.

N-15 enrichment in NH_4^+ was slightly increased for nonfumigated soil after 10-day incubation compared with N-15 enrichment in this fraction before incubation. Fumigation increased N-15 enrichments in NH_4^+ dramatically. CFIT releases a biologically meaningful, immediately available organic N flush. For NO_3^- in the soil, crop, date, and interaction between crop and date, fumigation effects were significant ($p < 0.01$). Interactions of fumigation x treatment was significant ($p < 0.1$), but interactions of date x fumigation, and fumigation x crop x date were not significant ($p > 0.1$). N was mineralized and further nitrified to NO_3^- for nonfumigated soil during 10-day incubation, while NO_3^- decreased in fumigated soils after a 10-day incubation. N-15 enrichment of NO_3^--N was 2- to 3-fold that of the NH_4^+ form before CFIT. Although NO_3^- accumulated in nonfumigated soil during incubation, the N-15 enrichment had a tendency to decrease. This indicates a non-labelled N source was released or nitrified to NO_3^-. However, N-15 enrichment in nitrate increased in fumigated soil after incubation although the total quantity decreased. Therefore, a N-15 labelled N source had contributed to this increase, or the enrichment was due to preferantoal loss of N-14.

N-15 excess in microbial biomass N was calculated with the three means using N-15 abundance in the atmosphere as a reference (Junk and Svec 1958). N-15 excess increased over time for the four treatments except for canola on July 24. The increase was 66, 42 and 41% in faba bean; -4, 19 and 117% in canola; 96, 44 and 20% in barley and 22, 72 and 25% in summer fallow. Estimated N-15 excess was slightly lowered when NH_4^+ in fumigated soil was taken solely to calculate biomass N than when the NH_4^+ in the nonfumigated control in 10 days incubation was subtracted (Table 4.2). Percentage of underestimation was 2-3, 2-3, 1-2 and 1-2% for faba bean, canola, barley and summer fallow, respectively. N-15 excess was lowest when both NH_4^+ and NO_3^- of fumigated and nonfumigated samples were used in the calculation. In this case, the underestimation was 7-39, 2-15, 0-10 and 3-28% for faba bean, canola, barley and summer fallow.

Quantities of N-15 in microbial biomass fraction in the 10-20cm depth were only 3 to 10% as much as in 0-10cm for all of the four treatments (Table 4.3). Atom % excess N-15 had a similar trend as for quantities of N-15, ranging between 6 and 17 % as great in the 10-20cm depth as in 0-10cm.

Table 4.2 N-15 excess (%) in flush N fraction in 0-10cm depth

Crop	Jul 8	Jul 24	Aug 19	Sept 1
Faba bean	0.23687	0.39233	0.55540	0.91508
Canola	0.29575	0.28373	0.33730	0.73268
Barley	0.18534	0.36260	0.52069	0.62673
Summer fallow	0.20382	0.24921	0.42767	0.53326

Table 4.3 Quantities of N-15 incorporated into fush N fraction over depth on the last sampling (ng ^{15}N g^{-1} soil)

Treatment	0-10	10-20cm
Faba bean	224.76	21.45
Canola	185.80	9.97
Barley	149.13	4.59
Summer fallow	113.35	7.82

4.3.3 Extractability ratio

Extractability Ratio (ER) is one way to characterize the sources of N (Legg *et al*. 1971; Juma and Paul 1984).

ER = (labelled N extracted /total N extracted)/(labelled total N/total N) (3)

It can be simplified to

ER = (atom%^{15}N abundance of extracted N / atom %^{15}N abundance of total N) (4)

The lower limit is zero and could be obtained if no labelled N were extracted. An ER > 1 implies that N compounds extracted are relatively enriched in N-15 compared with enrichment of total N in soil. Generally, the ER of NH_4^+-N was higher than that of NO_3^- when soil is tested without incubation. There was a decreasing trend for NH_4^+-N during the growing season, but not for the NO_3^- form. After a 10-day incubation of nonfumigated soil, ER was lower in the NH_4^+ fraction while it increased in the NO_3^- fraction. There was a consistent increase of ER in Fn throughout the growing season This follows from the pulse labelling technique used in this study, whereas N-15 in NH_4^+ before fumigation did

not present this trend. In other words, CFIT gives a better understanding of immobilized N and origin of the released N form in soil.

ER of Fn calculated with Equation 3, in which the flush was the NH_4^+ in fumigated and nonfumigated soils after 10 days incubation increased as growing season proceeded. The exception was under canola where ER decreased by 6 and 2% on the 2nd and 3rd samplings (Table 4.5). ER was generally higher in faba bean than that in the other three and lowest in the summer fallow. The percentage increase between adjacent sampling times was 19, 17 and 35% in faba bean; -6, 5 and 47% in canola; 28, 16 and 8% in barley; and 4, 27 and 11% in summer fallow.

Table 4.4 Excess N-15 (%) in flush N of depth (0-10, 10-20cm)

Treatment	0-10	10-20cm
Faba bean	0.91508	0.15619
Canola	0.73268	0.07541
Barley	0.62673	0.04079
Summer fallow	0.53326	0.06461

Table 4.5 Extractability ratio calculated from flush N in 0-10cm

Treatment	Jul 8	Jul 24	Aug 19	Sept 1
Faba bean	1.52	1.81	2.12	2.86
Canola	1.70	1.59	1.67	2.45
Barley	1.40	1.79	2.08	2.25
Summer fallow	1.41	1.47	1.86	2.06

4.4 Discussion

4.4.1 Soil Microbial biomass C and respiration C

Microbial biomass C estimated in faba bean plots was higher than that in canola, barley or summer fallow by 44, 39, and 167% on average of the four samplings, but canola and barley seemed to have similar microbial biomass. Faba bean supported a much higher microbial biomass than any of the other treatments. Summer fallow had the lowest

biomass, which may be due to the shortage of energy supply for microbial development.

Barber and Martin (1976) reported 18-25% of the total dry matter production of barley and wheat may be released by roots, which corresponded to 12-18% of the photosynthetically fixed C. Barber and Lynch (1977) observed that microorganisms on and around the roots could enhance the release of substrates. The cause may be both directly by the production of substrates which stimulate the process or indirectly by utilizing the exudates and preventing their build-up in solution, thus increasing outward diffusion. Martin (1977) used $^{14}CO_2$ labelled wheat and found that 39% of ^{14}C translocated to the root was lost as root exudates, root lysates, mucigel, cell wall residues and intact plant cells. Allocation of assimilated C from tops to roots could be as high as 50-85% for tallgrass (Dahlman and Kucera 1968); similarly, 15-25% was found in roots and shoot bases and 17-25% was lost by underground respiration from wheat (Warembourg and Paul 1973). Sauerbeck and Johnen (1977) separated CO_2 output from soils into soil respiration, root respiration and root decomposition respectively. They concluded that rhizodeposition of organic matter during growth of wheat exceeded the mechanically separated roots by about 20%.

Root dry matter of faba bean was 3 times as much as that in canola or barley whereas no significant difference between canola and barley was observed (Chapter 2). In contrast, microbial biomass of faba bean plots was 39-44% higher than that in canola or barley plots. Major differences exist for total C reduced during photosynthesis and thereafter the microbial population established between legume (faba bean) and nonlegumes (canola and barley). Reduced C is a primary limiting factor for microflora developing in an oligotrophic environment, such as soil (Tateno 1988). Martin (1975) found that about 15% of the labelled material in the leachates behaved as neutral sugar, the remainder as charged complexes. Monreal *et al.* (1981) observed that water soluble C in an Andept and a Mollisol was low relative to that needed for microbial maintenance and growth. The concept of maintenance energy was brought up again by Mallette (1963) and the theory was evaluated with *Escherichia coli* (Marr *et al.* 1963). In culture conditions, a maintenance coefficient of 0.07 g glucose (g dry wt)$^{-1}$ h^{-1} has been obtained (Pirt 1966). In contrast, water-soluble C in soil was only 20 μg g^{-1} soil (Dinwoodie 1988). It should be kept in mind that maintenance energy of soil microorganisms has not yet been quantified and it is only the general concept that is applied here for further consideration. Despite the losses of root exudates which are N rich compounds and materials, symbiotic N fixation in faba bean provided external N input into the plants and may enhance faba bean photosynthesis and

development, and consequently the flourishing of microbial population.

Microbial biomass C accounted for 2.9, 2.0, 2.1 and 1.1% of soil organic carbon for faba bean, canola, barley and summer fallow and these values of cropped soils are consistent with the 2-3% reported from mineralization-immobilization study on a Black Chernozemic soil in Saskatchwan (Paul and Juma 1981) and the results from the long-term forage rotation plots at this site in Alberta (McGill *et al.* 1986) except for faba bean. Biederbeck *et al.* (1984) reported 2.4% of soil organic carbon was contributed from microbial biomass C in a 12-year continuous wheat rotation receiving P only and 1.1% of the soil organic C in a fallow-wheat rotation in Saskatchewan. Jenkinson and Powlson (1976a) showed that stubbled soil contained considerably more biomass C than arable soil and the biomass constituted a higher proportion (3.7%) of the total soil organic C.

Because the microbial community in a soil environment changes in response to crop species, the relatively higher biomass C in the faba bean plot is a direct indication of a microbe-crop interaction. Lynch and Panting (1980a) observed a correlation between soil microbial biomass and root growth and rooting density of the crop. Foster and Rovira (1976) found that older roots of wheat at the flowering stage showed consistent development of microorganisms both in the rhizosphere and in the outer cortical cells and cell walls.

Respiration CO_2 evolved during 10 days incubation of the nonfumigated soil can be used as an estimate of potential soil biological activity. Respiration C of soil from canola and barley plots did not differ significantly between 128.8 - 132.9 $\mu g\ C\ g^{-1}$ soil, but fallow plot had lower respiration C 110.9 $\mu g\ C\ g^{-1}$ soil. These data were in good agreement with the results reported for soil under barley (Klemedtsson *et al.* 1987). Surprisingly, respiration C from soil in faba bean plots was even lower than that in summer fallow whereas microbial biomass C of the faba bean plots was generally the highest among all treatments. This suggests that the large number of viable microbes present in cropped soils does not give information on metabolic activity or state of these microorganisms. Nannipieri *et al* . (1978) showed that using one or two indices to estimate microbial biomass activity is too simplistic and not sufficient for interpreting the role of microorganisms in soils. Dinwoodie (1988) reported 43% of the microorganisms in the Gray Luvisol cropped to barley were active. Root exudates stimulate population increases but most of these organisms are either dormant or starved due to lack of available energy supply (Lynch and Panting 1980a).

Microbial biomass C and respiration C decreased in all treatments with increasing soil depth from 0-10cm to 10-20cm. Similar trends were obtained by Dahlman and Kucera (1968) using ^{14}C radioactive tracer. Three results parallel allocation of assimilated C to plant roots in various depths. The decrease in soil organic matter and available carbohydrate is a limiting factors for microorganism development. Limitation of root biomass in lower depth and exudation mainly from the basal regions of the root prevent microflora development in soil (Clark and Paul 1970). Close relationship between energy supply and crop growth had already been reported (Christie *et al.* 1986; Powlson *et al.* 1987).

4.4.2 Flush N released following fumigation

Fumigation released NH_4^+-N as evident from the 20-90 fold increase of NH_4^+ from our field samples following fumigation. The trend is in good agreement with initial studies by Jenkinson and Powlson (1976a) and a more detailed study on 37 soils at 3 depths by Brookes *et al.* (1985). Results from Brookes *et al.* (1985) indicate the flush of N released accumulated as NH_4^+ was in the range 10-100 over the control. Brookes *et al.* (1985) proposed this fraction of NH_4^+ probably derives from cytoplasmic components of the soil microbial biomass.

A small decrease of NO_3^- was observed following chloroform fumigation, and confirmed that nitrifying bacteria were killed during the fumigation process and had not recovered because there was no increase in NO_3^- in soil after 10-day incubation.

Treatment effects on NH_4^+ release were not significant ($p < 0.1$) though sampling effects were ($p < 0.1$). Our results may be due to the sampling depth 10cm and the time for samples storage. Significant differences may be obtained when shallow surface samples are compared. Further nitrogen immobilization during biomass determination should be considered in such interpretations (Nannipieri 1984). Storage of samples for 24 hours before measurement produced insignificant changes in the microbial biomass, but effects of further storage of soil samples on microbial biomass estimated is not available from the literature. Lynch and Panting (1980a) observed that sieving soil reduced the estimated microbial biomass , thus the method to measure soil microbial biomass is not an absolute estimation. A further limitation is the uncertainty of fumigation efficiency and therefore the Kc value.

The chloroform fumigation technique is based on a series of assumptions

(Jenkinson and Ladd 1981). One is that mixed populations of microorganisms killed decompose to an equal extent in various soils. Furthermore, Kc and Kn values may be applied to qualitatively and quantitatively different biomasses. If this assumption is true, then a correlation between flush of carbon and flush of N during the 10-day incubation should be observed. In this study, there was generally very poor correlation between them for barley, faba bean, and fallow. Only in the case of canola did the correlation reach 0.60. In soil environments, fungi appear earlier than bacteria and their compositions are distinctly differnet. The heterogeneity of substrates (killed microbial biomass) (McGill *et al.* 1981) for surviving bacteria after fumigation makes quantitatively and qualitative estimation of microbial biomass difficult. The C/N ratio is one of the governing factors in decomposition processes and the cytoplasmic component is normally mineralized more quickly than cell walls, which often have a higher C/N ratio. The C calculated from CO_2 evolved and N mineralized are the portion of microbial cell components that decomposed in a 10-day incubation. Therefore, "microbial biomass C" and "microbial biomass N" may not reflect the partially recalcitrant nature of microbial cellular structures, especially for fungal hyphae. Although partitioning bacterial and fungal biomass may be desirable, lack of reliable information about the details in regards to bacterial and fungal biomass dynamics makes modelling this separate dynamics of bacteria and fungal biomass quantitatively impossible.

Microbial biomass N can be calculated in several ways (Paul and Voroney, 1984), including using a control of 0-10 day or 10-20 days, or no control at all for the fumigated soils. Fn, instead of microbial biomass N, has been reported (McGill *et al.* 1986). Three calculations were conducted to estimate the flush N and results of quantities of N-15 incorporated into the microbial biomass using Equation 2 were presented (Table 4.1). In this calculation, Fn was taken as the NH_4^+ released by chloroform fumigation minus the amount of NH_4^+ in the nonfumigated control during 10 days incubation. Among the several means to calculate the microbial biomass N, NH_4^+ is the key cation to be analyzed and estimation from NH_4^+ gives consistent results even though the control is eliminated.

The N-15 excess in microbial biomass fraction is lower in summer fallow than that in soil cropped to faba bean, canola or barley. Results suggested either a higher turnover rate was operating in cropped soil or N-15 labelled exudates were generated for microbial growth. Faba bean is a legume crop and N fixation can be as high as 184 - 198 kg N ha^{-1} yr^{-1} (Chapter 3). N-15 labelled NH_4^+ or NO_3^- must be assimilated then distributed among various components of a crop depending on demands, therefore the higher N-15 excess found in microbial biomass must be associated with interactions of crop root in soil and

added N-15 remaining in the inorganic N pool. Unless highly N-15 enriched compounds were released to microorganisms in soil rhizosphere or the turnover rate is high then biomass N would not be so enriched under a legume. From chapter 3 , about 18-22% N in roots originated from the atmosphere and 79% for N in tops, so N in roots had high N-15 excess and so will the metabolites released or exudated. However, this single source could not explain the higher N-15 excess. In addition, turnover rate must be considered to account for the high N-15 excess found in soil cropped to faba bean.

In 10-20cm depth, N-15 excess was higher in faba bean plot, followed by canola and summer fallow, while barley had the lowest N-15 (Table 4.3). This may be because the maturity of barley had been reached before the last sampling and canola was near fully mature, but faba bean was in grain-filling stage. Because N-15 was applied to surface soil four times, quantities and excess N-15 would be partially the result of N-15 movement down to the lower depth. Apart from this, physical root elongation and soil animal movement can also contribute to the enhanced N-15 movement in soil. This could be due to root exudation of N-15 enriched polymers into the rhizosphere soil and microorganism feed on these available energy sources thereafter. Also the physical action of roots during growth, together with root channels may accelerate mass transfer.

4.4.3 Extractability ratio

ER was and is still used as an index of N mineralized from the soil organic matter fractions (He *et al.* 1988; Juma and Paul 1984; Legg *et al.* 1971). In our study, it has been shown that microbial biomass fraction N calculated from Equation 3 is the best estimates for microbial biomass N, the biologically meaningful fraction; therefore, the ER calculated for this fraction would obtain the same meaning. Results showed that ER increased concurrent with addition of N-15 in the growing season. Juma and Paul (1984) argued that chloroform fumigation and aerobic incubation caused almost identical newly immobilized N-15 to be released. During 10 days incubation of this Gray Luvisol, NO_3^- from nonfumigated soil was more enriched in N-15 than the NH_4^+ released by fumigation. The magnitude was around 40% (data not shown).

Based on all the variables obtained through this study, an attempt was made to correlate microbial biomass C and N of the same origin. Since the substrate carbon source and species of microorganisms were not determined, poor correlations between microbial biomass C and Fn would not be interpretated correctly. Further statistical analyses were conducted to clarify the source of Fn. Biomass C is negatively correlated to NO_3^-, NH_4^+

before fumigation, after fumigation and incubated, nonfumigated but incubated, and also the sum of these sources. The higher correlation coefficients were those for NO_3^- at time zero, mineral N at time zero, NO_3^- after CFIT, mineral N of nonfumigated but incubated soil, and N flush. 94% variation can be explained by four components and 98% by 5 components. The first principal component, which accounts for 50% of the total variation, is highly and positively correlated to three forms of NO_3^- and three forms of mineral N, but not NH_4^+. Therefore, this component is actually associated with soil properties, such as original N status. The second component, accounted for 23%, is related to all three forms of NH_4^+ and Fn. This component is actually the one associated with CFIT effect and flush of NH_4^+.

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CHAPTER 5. SYNTHESIS

5.1 Summary

Applied N-15 enriched nitrogen was taken up by plants and distributed in crop tops and roots. N-15 excess in various N-containing components follows: crop tops > crops roots > flush N > crop residues (0-10cm). Inotganic nitrogen pool had low N-15 excess and N-15 excess was higher in nitrate. The general pattern over the four samplings was a bell-shape curve,N-15 excess increased till the 3rd sampling and then decreased on the 4th sampling. Crop residues on soil surface had higher N-15 excess than that in the soil. N-15 excess of total soil itrogen was the lowest one among various components determined.

Results of this study have suggested both canola (*Brassica napus* L.) and barley (*Hordeum Vulgare* L.) can be used as reference crops for the N-15 dilution method to quantify dinitrogen fixation in faba bean (*Vicia faba* L.). Total difference method in this study was comparable to N-15 dilution for quantification of dinitrogen fixation in faba bean.

Throughout the four samplings, dry matter production of faba bean differed from that of canola and barley significantly after faba bean entered the stage of regenerative growth ($p < 0.01$). There were no significant differences in dry matter production among the legume (faba bean) and nonlegume (canola and barley) during stage of vegetative growth. On the last sampling (September 1), above ground C in faba beans totalled 4.4 t C ha^{-1} compared with 1.6 t C ha^{-1} in canola and 1.4 t C ha^{-1} in barley. C accumulated in roots totalled 1.1 t C ha^{-1}, 300 kg C ha^{-1} and 337 kg C ha^{-1} in faba bean, canola and barley, respectively.

A significant amount of the N assimilated in faba bean stands comes from symbiotic dinitrogen fixation, the quantity estimated during 1987 was 183-199 kg N ha^{-1} in tops and 18-22 kg N ha^{-1} in roots within 27cm depth at Breton. Faba bean was not fully matured by the final harvest and may not have completed dinitrogen fixation. Atmospheric N in faba bean accounted for 65-80% of the total N accumulated. Maximum symbiotic dinitrogen fixation rate was estimated to be 4.0-4.7 kg N ha^{-1} day^{-1}.

Microbial biomass C and respiration C in soils responded to crop species differently. Microbial biomass C estimated in faba bean plots was higher than that in canola, barley or summer fallow by 44, 39 and 167% on the average of four samplings. A peak was observed for canola, barley and summer fallow around July 24, but it was not

evident for faba bean. Flush N was not significant upon treatments, and N-15 incorporated into the flush N fraction and N-15 excess varied with treatments. Using NH_4^+ in fumigated less the nonfumigated soil gives the best estimation of flush N.

Fate of applied N-15 urea

Urea is hydrolyzed to carbon dioxide and ammonia by the enzyme urea amidohydrolase which acts on nonpeptide C-N bonds in linear amides (Bremner and Mulvaney 1978). Ammonia is susequently hydrolyzed to ammonium, which can be: 1. taken up by plants and become components of crop dry matter, shoots and roots; 2. nitrified by microorganisms to nitrate and thereafter subject to losses by leaching and denitrification; 3. immobilized by microorganisms and incorporated into residual organic material remaining in soil or on the surface of soil; 4. incorporated into soil organic matter becoming a component of soil total N; 5. immobilized by microorganisms and recycled in this components as microbial biomass; and 6. present in the inorganic N pool in a small quantity. All these components have been determined in this study, but only the N and N-15 in plant tops and roots, and those in microbial biomass are reported in Chapter 1 and Chapter 3. N-15 excess in each of these components is a direct indication where applied N-15 has gone and the data are presented in Table 5.1.

N-15 enriched urae-N was taken up and distributed between crop tops and roots, and the N-15 excess in crop tops was higher than that in roots of canola and barley. Faba bean roots were slightly more enriched in N-15 than tops. Crop residues from previous cultivation on soil surface immobilized applied N-15 as well. Flush N from microbial biomass is another sink for N-15 applied. Generally, nitrate is more enriched in N-15 than ammonium, especially for summer fallow. Ammonium in this soil condition may be the limiting factor for substrate transformation biochemically from ammonium to nitrate.

Table 5.1 N-15 excess of applied N-15 in various components at Breton in 1987

Crop	July 8	July 24	August 19	September 1
		Crop tops		
Faba bean	0.291	0.772	0.528	0.645
Canola	1.994	2.973	2.982	4.236
Barley	1.854	2.851	4.082	3.364
		Crop roots		
Faba bean	0.390	0.779	0.557	0.767

Canola	0.824	1.271	1.432	1.722
Barley	0.667	1.039	1.042	1.458
		Flush N(0-10cm)		
Faba bean	0.237	0.392	0.555	0.915
Canola	0.296	0.284	0.337	0.733
Barley	0.185	0.363	0.521	0.627
Summer fallow	0.204	0.249	0.428	0.533
		NH_4^+ N(0-10cm)		
Faba bean	0.079	0.077	0.267	0.020
Canola	0.076	0.063	0.120	0.017
Barley	0.054	0.070	0.146	0.011
Summer fallow	0.071	0.073	0.153	0.015
		NO_3^- N(0-10cm)		
Faba bean	0.783	1.070	0.853	0.137
Canola	0.721	0.405	0.452	0.132
Barley	0.363	0.554	0.611	0.094
Summer fallow	1.589	1.569	1.249	1.842
		Soil total N(0-10cm)		
Faba bean	0.031	0.053	0.069	0.081
Canola	0.022	0.041	0.055	0.083
Barley	0.027	0.040	0.060	0.075
Summer fallow	0.037	0.057	0.061	0.071
		Crop residues(0-10cm)		
Faba bean	0.135	0.258	0.357	0.443
Canola	0.114	0.234	0.291	0.459
Barley	0.092	0.176	0.283	0.419
Summer fallow	0.071	0.170	0.208	0.336
		Crop residues on soil surface		
Faba bean	0.248	0.555	0.650	0.882
Canola	0.291	0.616	0.788	1.276
Barley	0.243	0.656	0.402	1.336
Summer fallow	0.420	0.903	1.142	1.417

N supply to crops

Crop is a major sink for inorganic nitrogen in the soil. Difference in crop species may result in variations of the amount of N assimilated and distribution of N among above and below ground components. Crop yields are often directly proportional to the N released from organic matter. The other nutrients are also important but N is required in much larger amount and is more likely to be deficient (Campbell 1978). Plant uptake of N

tends to precede maximum N mineralization rate revealed on barley, but the two processes are reasonably synchronized (McGill and Myers 1987). Faba bean and barley tops had the highest N-15 excess among all the fractions analyzed, crop roots, flush N, soil total N, ammonium, nitrate, crop residue from previous cropping, and such residues on soil surface. N-15 excess in faba bean tops was lower than that in canola or barley tops. Faba bean differs from canola and barley in that the former can assimilates dinitrogen from the atmosphere during its development, especially under condition where soil nitrogen is deficient and rhizobium is in presence in the soil. N-15 excess in faba bean was lowered because dinitrogen from atmosphere had a very low abundance. Contrarily, canola and barley depends on soil N for their physiological development. If soil N is limited and mineralization of soil organic matter can not meet crop demands, then crop growth is limited by nitrogen supply. However, the N from soil would have a higher abundance.

Crops accumulate dry matter by assimilating carbon dioxide at a rate which depends on the physical state of the environment and on the physiological state of the foliage (Biscoe *et al.* 1975). Environmental conditions and cultivation practices influence dry matter accumulation in the above and below ground components differently. Increasing mineral supply tends to increase shoot growth relative to root growth; increasing water supply has the same effect; increasing light intensity promotes root growth at the expense of shoot growth (Brouwer 1962). Since the roots depend on the shoots for their carbohydrate supply and since they are at a great distance from the leaves where the carbohydrates are produced, the latter apparently limit root growth more often than shoot growth. The availability of carbohydrates is closely associated with the N status of the crop. Since CO_2 assimilation is positively related to tissue N concentration and grain starch, accumulation is primarily dependent on current photosynthate (MacKown and Van Sanford 1988). A large amount of N is stored as amino acids and acid amides (asparagine and glutamine) during the early growth stages. Lignin and cellulose production is more important during aging than during the early vegetative stage.

Mineralization of soil organic matter is mediated by soil microorganisms and the latter are affected by environmental conditions and availability of substrates (Jasson 1958; Juma and McGill 1986; McGill *et al.* 1986). Energy supply to microorganisms is the driving and directing force in soil nitrogen metabolism. Inorganic nitrogen immobilization is a general and indispensable part of the nitrogen metabolism of mornal soil microflora. During the life cycle of wheat, leaves sequentially initiate senescence from the oldest leaf up to the flag leaf. As a leaf develops, the influx and export of N changes (MacKown and

Van Sanford 1988). N in dead shoots is transferred to the metabolic and structural components of standing live parts. In the case of death from aging and drying, the plant appears to conserve N (Clark 1977), so that the C/N ratio of dying shoots will be higher than that of live shoots. Generally, plants contain the same classes of compounds, but the proportions of each, which depend upon the species and maturity, may influence the degree and rate of decomposition. Herman *et al.* (1977) reported that increasing C/N ratio or lignin content reduced carbohydrate loss and total C loss during decomposition but increased the rate of lignin loss. The organic matter content of a soil reflects the balance between additions and removals. Cultivation causes changes both in the rate of addition and the rate of decomposition (Juma and McGill 1986). Mineralization of N from plant material is mediated by microbial community, and the C/N ratio of the material determine whether N is mineralized or immobilized. Soil organic matter is mineraliized at a relatively constant rate for each of the soil under its environmental condition. Soil characteristics revealed a dynamic equilibrium between organic carbon and nitrogen in the soil determined by the activities of the microbial population. When the equilibrium was attained, C and N were mineralized in a constant proportion. If the ratio was increased by addition of carbonaceous material, dereased nitrogen mineralization could be expected, and if the ratio was decreased by addition of highly nitrogenous materials, incraesd nitrogen mineralization would result.

Evidently, the C/N ratio only provides a rough approxamation of the really important factor, the available energy-available nitrogen ratio. The different constituents of the organic materials concerned are not equally available for microbial decomposition (McGil, *et al* . 1981). Thus materials having the same C/N ratio may have quite different effects upon the mineralization-immobilization condition in the soil with regard to the supply of plant-available nitrogen.

Root ecology and root physiology are now the two main fields in root research. Root dynamics and its dry matter production have been hindered by the methodology which is available for extracting crop roots from soil. Bohm (1979) considered field condition root research is a step-child of science due to method primarily. The rapid development of root research in the last few years is leading more and more to a specialization in this area of science. Results from roots have provided more accurate data on nutrients in the root fraction, Kirchmann (1988) concluded white and red clovers are superior to other legumes as green manure because of larger amounts of N in roots as revealed by a washing technique. Hansson *et al.* (1987) state a higher proportion of total

biomass and production was found below-ground in the unfertilized barley compared to the fertilized barley. Nitrogen fertilization had a more pronounced effect on top than on root growth.

Plant roots constitute a major part of the soil ecosystem and are a main source of organic C material. A considerable amount of nitrogen mineralized during the growing season is used in plant production above and below ground. As soil dried, the small roots died and disappeared. Quantifications therefore are difficult or impossible. Using $^{14}CO_2$-fed wheat and barley, Barber and Martin (1976) found that 12-18% of the C fixed by young (3-week-old) cereals was released by the roots. Rovira (1969) observed that young plants may produce more exudates than older ones. Warembourg and Paul (1973) detected no ^{14}C in rhizospheres of wheat plants at heading and soft dough stage pulse-labelled for 3.5 days with $^{14}CO_2$. They attributed this to the short labelling time.

Root mass measurements require extensive labor during sampling, and during sample treatment, washing and fractionating. Consequently, root mass is often sampled only once when it is assumed to be at its maximum. Root mass does not increase at a constant rate during the vegetative growth, but instead the rate varies and root mass can even decrease. These fluctuations are caused by phenological changes in the plants or by environmental conditions (Hansson 1984). Root production in certain forest stands is about equivalent to leaf litter input (Heal and Ineson 1984).

The root-washer used in this study performed well on separation of plant materials (both live and dead) from soil. Though light clay was found in plant materials, separation between clay and roots (live roots and dead organic materials) was much easier than that of the latters. Since roots of barley and canola are very fine, error on these may be larger than on faba bean. It could be more pronounced for incompletion of separation of live from dead roots. Study of roots with large amount of soil from experiments is not recommended since the destruction to plots will affect later experiment and interpretation of soil data. Distribution of root C within depths (0-10, 10-20 and 20-27cm) was 86, 10 and 4% for faba bean; 84, 33 and 5% for canola; and 80, 15 and 5% for barley. Chi-square test showed that gross root vertical distribution patterns of these three crops did not differ significantly.

Environmental conditions, such as temperature, moisture and soil bulk density affect crop growth. The open-ended cylinders employed in our study may have restrained root development of the crops. This effect is more significant on faba bean than on canola

or barley based on one sampling at the end of experiment for crops inside and outside the cylinder. Comparing with faba bean outside, but adjacent to the cylinders, those inside were much smaller and thinner. Gray Luvisol is a low fertility soil for crop growth, and this may be more significant when no fertilizer was applied. It should be noted that the physical condition of soil has profound impact on crop development and growth. Therefore, manipulation on agricultural ecosystem is possible on controlling processes taking place in soil.

N from symbiotical dinitrogen fixation

N is an important nutrient to agricultural production. Symbiotic dinitrogen fixation has drawn more and more attention as information on N transformations in soil accumulated because symbiotic N fixation is an important input of N into the biosphere. Faba bean (*Vicia faba* L.) is a source of protein in developing countries and the reported amount of dinitrogen fixed varies between 54-209 kg N ha^{-1} yr^{-1} (Chapter 3; Dean and Clark 1977; Rice 1976; Richards and Soper 1982; Zapata *et al.* 1987). Various approaches are available for quantifying dinitrogen fixation in faba bean; among them are the total difference method, N-15 isotope technique (Danso 1988), acetylene reduction (Witty and Minchin 1988) and ureide assay. Unfortunately, results from different measurements vary widely. Acetylene reduction estimates of dinitrogen fixation for soybean grown in the field or under controlled environment conditions were about half those of the total difference assay (Smith and Hume 1987). Under Egyptian field conditions, the estimated average amount of atmospheric nitrogen fixed by faba bean is 135 kg N ha^{-1} yr^{-1} as measured by acetylene reduction (Abdel-Ghaffar 1988). Results obtained from our study were in a good agreement with those reported by Zapata *et al.* (1987) on total amount dinitrogen fixed and fixing rate. It will be meaningful to see if the results can be reproduced and comparable when experiments are conducted on a different type of soil instead of reproducing experiment on the quantitative aspects of dinitrogen fixation.

The basic assumptions of N-15 dilution were established by McAuliffe and coworkers (1958), but few attempts have been made to evaluate the validity of these assumptions. N-15 dilution is the only method that distinguishes the contributions to plant-N of soil, fertilizer and atmospheric nitrogen. Error from a mis-match between reference and fixing crop tend to be small at high values of fixation (Danso 1986; Weaver 1988). Wagner and Zapata (1982) suggested that the ratio of soil S-to-fertilizer S uptake by fixing and reference plants could be used as indices of relative uptakes of soil N and fertilizer N in soil labelled with $(NH_4)_2{}^{35}SO_4$. Labelling strategies fall into three broad categories: the

addition of fertilizer N, the addition of immobilized N, and the use of a residual label in the soil from previous tracer additions (Chalk 1985).

N-15 isotope technique is widely accepted for quantification of dinitrogen fixation in a legume and it depends on differences in isotopic composition of the sources of N available for plant growth (Phillips *et al.* 1986). The application of the principle of isotope dilution to estimate dinitrogen fixing has relied on the use of a non-fixing reference plant to assess the relative availability of soil plus fertilizer N over the growing period. However, evidence has accumulated indicating that several factors can interact to invalidate the assumptions in N-15 dilution method. Differences may arise from the small natural enrichment of N-15 in soil N, or from the addition of N-15 enriched or N-15 depleted materials to the soil. Although questions are frequently asked about the validity of employing a nonfixing crop, which may differ from the legume morphologically, accuracy of this method is highly praised (Rennie 1982). Since sophisticated equipment, such as a mass spectrometer is required to analyze samples, cost and availability of such a facility prevents some researchers from going into this field of study. However, Smith and Hume (1987) reported that the N-15 dilution and difference method estimates were not different within or between species of white bean (*Phaseolus vulgaris*).

Chalk (1985) reviewed N-15 isotope dilution and emphasized the widely held belief that indirect isotope methods will provide accurate quantitative estimates of dinitrogen fixation. On closing a workshop on Biological nitrogen fixation in Mediterranean-type agriculture recently, Mytton (1988) stated that the N-15 method is recommended for accurate quantification.

In this study, an attempt was made to see if the total difference method was comparable to N-15 isotope technique. Results showed that total difference method is comparable to N-15 dilution method on N fixed in faba beans on this soil. Mytton (1988) emphasized that international cooperation in research and training must be encouraged to further evaluate methods for quantification of dinitrogen fixation in various legumes. In doing so, data reported could be compared on a common basis of method used and soil type.

Driving force in N cycling

Soil microbial biomass is both a source and sink for nutrients and plays a very important role in nutrient cycling. Nutrient cycling and crop productivity are therefore

influenced by the amount and activity of soil microbial biomass. Measurements are essential and continue to be a problem. Comparisons between different methods: plate counting, direct microscopic techniques, and chemical techniques such as ATP measurement and CO_2 measurement after fumigation, show that the data obtained are rather inconsistent, with chemical techniques giving the highest biomass values (Paul and van Veen 1978). There is still a need for methods, which can be applied conveniently to obtain reliable and comparable biomass values in soil ecosystem studies. Soil microbial biomass is often quantified by the chloroform fumigation and incubation technique of Jenkinson and Powlson (1976a; 1976b). Various calculations were also available for the same fraction of soil organic matter as soil microbial biomass (Paul and Voroney 1984). Microbial biomass in soil is affected by various factors, cropping history (McGill *et al.* 1986), tillage, crop species (Barber and Martin 1976) and organic amendment. McGill *et al.* (1986) reported that quantity of microbial biomass was controlled by long term C additions whereas fluctuation of moisture controlled short-term biomass dynamics.

Conclusion

This study suggested that symbiotic dinitrogen fixation in faba bean can be estimated by the N-15 dilution technique with either canola or barley as a reference crop. The total difference method and N-15 dilution technique are equally important approaches in quantification of nitrogen fixation in faba bean on this Gray Luvisol at Breton. Between 183-199 kg N ha^{-1} yr^{-1} was fixed in faba bean tops before full maturity and 18-22 kg N ha^{-1} yr^{-1} was translocated from tops to roots using N-15 dilution technique. Microbial activity in soil affects C and N transformation, but reliable methods are needed. Ammonium was more sensitive to chloroform fumigation and incubation technique than both ammonium and nitrate were considered. Subtracting a nonfumigated control or not gives equally important information.

Implications for soil management

In the maintenance of a high fertility level in arable soils, their organic matter content requires special attention. Crop residues and organic manures have to be incorporated in the soil to compensate for decomposition losses and provide a continuous renewal of the organic matter content. In addition, the decomposition processes have to be controlled by a suitable crop rotation, providing a favorable balance between production of crop residues to be incorporated in the soil on the one hand, and tillage operation's stimulating decomposition on the other. Production and carefull utilization of crop residues

and organic manures is of fundamental importance in all methods of soil conservation and restoration.

Faba bean is a promising legume for the quantity of dinitrogen fixed in sustainable agriculture and its living stands are important for forage use. One limitation of using faba bean as forage or green manure is the seeds needed for seeding is at a larger amount, and may not be economically feasible. At place where faba bean seeds can be harvested, applying faba bean in rotation can be a beneficial one.

Much consideration is being given at the present time, especially in some of the underdeveloped tropical and subtropical countries, to the further development of multiple cropping or intercropping systems to enhance agricultural production. These techniques are designed to make full use of both the land available for cultivation and of the growing period of the crop.

Although the basic practices of intercropping are often centries old, it is only recently that they have become the subject of intense interest by the scientific community concerned to maximize crop production. Nitrogen fixing species clearly play an essential role in the maintenance, by biological means, of the fertility cropped land as a result of the decay of plant residues and in some cases by excretion of nitrogen-rich root exudates.

Future research

Research of a long-term nature is in progress in many parts of the world to obtain the maximum productivity from legume applied systems by defining such factors as the timing of cropping intervals, the most suitable combinations of nitrogen-fixing and non-nitrogen-fixing species for intercropping systems, and the optimum plant spacings within such systems, and also to determine what proportion of the nitrogen fixed by an intercropped nitrogen-fixing species may become available to associated plants or to enhance soil fertility following the decay of plant residues.

The need for accurate measurement of dinitrogen fixation in legume must be thoroughly assessed. In general, empgasis should be placed on simple indirect methods (Mytton 1988). Yield comparison between legumes and comparable non-legumes are also valuable. Measurement of simple parameters such as dry weight, grain yield, and total nitrogen are highly recommended and may also be of agronomic value.

Nitrogen transfer from legumes to companion crop needs more research. Evidence

for substantial and rapid movement is sparse. However, the information is important for rational development of inter-cropping legumes with non-legumes and is also relevant to pasture systems.

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CHAPTER 6. APPENDICES

Appendix 1. Shoot dry matter of crops at Breton plots in 1987*

Crop	1st	2nd	3rd	4th sampling
Barley	5.10	8.77	8.13	12.00
	2.66	8.82	11.39	10.81
	4.65	5.93	9.34	12.02
	5.01	7.86	9.29	9.03
Canola	2.76	7.05	12.05	11.14
	2.77	6.69	11.19	12.89
	3.36	7.40	10.18	10.92
	4.78	5.81	9.71	8.98
Faba bean	4.83	6.58	19.47	28.49
	5.19	9.28	28.31	29.80
	6.68	12.76	17.05	41.11
	8.36	9.15	50.64	23.97

* Dry matter(g) was actual raw data in area of 20cm diameter cylinders.

Appendix 2. Root dry matter of crops at Breton plots in 1987*

Depth (cm)	1st	2nd	3rd	4th sampling
		Barley		
0-10	0.85	0.66	0.48	1.79
	0.60	0.63	1.20	1.63
	0.80	0.51	0.92	0.27
	1.35	0.82	0.93	0.68
10-20	0.15	0.07	0.07	0.42
	0.14	0.06	0.09	0.06
	0.17	0.09	0.05	0.21
	0.12	0.04	0.08	0.24
20-27	0.05	0.09	0.02	0.05
	0.03	0.02	0.04	0.03
	0.07	0.10	0.07	0.10
	0.06	0.05	0.02	0.08
		Canola		
0-10	0.49	0.45	1.95	0.81
	0.53	0.35	0.66	1.11
	0.79	0.56	0.74	0.85
	0.61	0.64	0.81	1.15
10-20	0.08	0.21	0.18	0.09
	0.11	0.09	0.05	0.17
	0.14	0.11	0.13	0.15
	0.21	0.05	0.08	0.11
20-27	0.04	0.05	0.02	0.07
	0.02	0.03	0.07	0.07
	0.04	0.06	0.03	0.07
	0.06	0.02	0.05	0.07
		Faba bean		
0-10	1.66	1.92	2.13	2.92
	2.18	2.56	4.35	5.89
	1.15	1.71	1.96	3.58
	1.46	1.50	6.13	3.32
10-20	0.12	0.45	0.34	0.59
	0.38	0.20	0.54	0.60
	0.33	0.33	0.16	0.44
	0.11	0.24	0.88	0.48
20-27	0.11	0.03	0.05	0.18
	0.17	0.04	0.21	0.24
	0.13	0.09	0.08	0.17
	0.06	0.04	0.17	0.16

* Dry matter(g) was actual raw data in area of 20cm diameter cylinders.

Appendix 3. Organic residues at Breton plots in 1987*

Depth (cm)	1st	2nd	3rd	4th sampling
		Control		
0-10	10.51	5.49	7.90	6.03
	6.64	6.73	8.35	9.71
	8.46	5.77	5.24	6.98
	6.03	5.12	6.04	6.02
10-20	4.82	1.65	2.00	2.19
	3.48	3.10	4.71	3.62
	3.82	3.03	4.30	1.45
	1.01	1.57	2.22	2.78
20-27	0.73	0.10	0.23	0.20
	0.11	0.46	0.27	0.93
	1.01	0.30	0.11	0.09
	0.11	0.52	0.12	0.16
		Barley		
0-10	9.75	8.20	12.31	7.73
	5.88	6.43	6.24	8.31
	4.89	6.58	6.73	3.90
	9.41	7.31	9.13	5.60
10-20	4.46	3.93	7.53	7.21
	3.84	2.43	0.73	1.99
	2.71	1.81	2.93	2.78
	4.10	3.67	3.15	3.47
20-27	1.58	0.52	4.32	0.15
	0.24	0.40	0.67	0.07
	0.13	0.18	0.10	0.24
	0.81	0.15	0.23	0.08
		Canola		
0-10	11.04	6.81	6.54	8.33
	9.88	6.30	5.28	7.10
	7.79	3.72	4.70	4.90
	8.12	9.11	5.13	7.70
10-20	2.73	4.33	4.49	2.72
	1.61	2.78	1.84	3.65
	3.34	1.30	1.42	1.88
	4.09	2.95	3.32	1.40
20-27	0.13	0.77	0.31	0.11
	0.48	0.27	0.32	0.21
	0.11	0.20	0.09	0.21
	0.09	1.13	0.36	0.14

(continued)

		Faba bean		
0-10	15.03	8.79	5.31	8.99
	8.99	8.29	5.67	6.37
	9.75	9.05	8.05	5.86
	6.73	6.30	4.52	7.44
10-20	4.21	4.80	2.34	4.19
	4.09	1.58	2.43	2.22
	3.75	4.38	3.14	4.56
	2.98	4.78	2.92	1.98
20-27	0.14	0.21	0.11	0.24
	0.57	0.10	0.17	0.09
	0.10	0.27	0.14	0.52
	0.13	0.48	1.16	0.21

* Dry matter(g) was actual raw data in area of 20cm diameter cylinders.

Appenidx 4. Fractionation of above ground components of crops*

Components	1st	2nd	3rd	4th sampling
		Barley		
Stems	0.46	2.64	1.59	2.14
	0.95	2.27	2.15	2.07
	0.93	1.48	1.95	2.32
	0.99	2.09	1.89	1.66
Live leaves	0.76	0.56	0.08	0.00
	1.15	0.57	0.00	0.00
	0.93	0.37	0.00	0.00
	1.06	0.44	0.00	0.00
Dead leaves	0.28	0.78	0.68	0.91
	0.90	1.06	0.91	1.01
	0.79	0.75	0.63	1.08
	0.66	0.89	0.72	0.52
Sheaths	1.11	1.35	1.12	1.39
	2.09	1.63	1.89	1.29
	1.94	1.02	1.47	1.80
	2.25	1.44	1.36	1.30
Heads		3.02		
		2.79		
		1.96		
		2.69		
Seeds			3.84	6.04
			5.25	5.31
			4.25	5.38
			4.24	4.54
Husks			0.90	1.30
			1.19	0.99
			0.95	1.13
			1.06	0.85
		Canola		
Stems	0.98	4.65	7.48	5.59
	1.14	4.33	6.96	7.20
	1.18	4.70	6.27	6.51
	2.39	3.72	4.94	5.36
Leaves	1.73	2.09	1.34	0.00
	1.58	2.12	0.52	0.00
	2.15	2.36	0.56	0.00
	2.34	2.09	0.56	0.00
Shells			2.64	3.14
			2.83	3.69
			2.56	2.68
			2.15	2.36

Seeds			0.67	2.30
			0.98	1.83
			0.85	1.52
			1.15	1.17
		Faba bean		
Stems	1.24	3.91	9.81	17.04
	1.43	2.25	13.69	14.38
	1.63	3.75	9.72	19.56
	3.08	6.18	25.58	14.57
Leaves	3.51	4.83	6.87	10.83
	3.61	3.92	9.07	8.37
	5.00	5.03	5.87	9.35
	5.23	5.88	14.37	8.54
Shells			2.33	0.36
			4.21	6.80
			1.12	11.90
			3.20	0.65
Seeds			0.34	0.20
			0.91	1.70
			0.28	3.88
			0.48	0.00

* Dry matter(g) was actual raw data in area of 20cm diameter cylinders.

Appendix 5. N content (%) in above ground components of crops

Components	1st	2nd	3rd	4th sampling
		Barley		
Stems	0.658	0.443	0.797	0.561
	0.758	0.434	0.481	1.358
	0.643	0.451	0.446	1.254
	0.489	0.356	0.424	1.502
Live leaves	2.921	2.205		
	3.311	1.887		
	2.751	1.965		
	2.234	1.914		
Dead leaves	1.294	1.681	2.556	1.819
	1.416	1.137	1.284	0.514
	1.251	1.029	1.142	0.591
	1.116	1.096	1.162	0.599
Sheaths	1.656	1.562	2.090	1.374
	1.848	1.430	1.099	1.038
	1.497	1.472	0.966	0.879
	1.439	1.263	1.003	0.986
Heads		2.419		
		1.975		
		2.041		
		1.932		
Seeds			1.886	1.786
			1.765	1.738
			1.475	1.755
			1.727	1.651
Husks			1.361	0.802
			0.865	0.754
			0.891	0.560
			0.782	0.759
		Canola		
Stems	1.731	0.952	0.846	0.596
	1.632	1.079	0.629	0.691
	1.532	0.846	0.601	0.449
	1.322	1.059	0.644	0.731
Leaves	2.733	2.867	1.911	
	3.031	3.109	1.805	
	2.577	2.987	1.906	
	2.398	3.025	1.836	
Husks			1.295	0.766
			1.209	0.922
			1.151	0.781
			1.110	1.216

Seeds			2.846	2.979
			2.704	2.978
			2.728	2.661
			2.739	2.947
		Faba bean		
Stems	1.891	1.518	1.316	1.154
	1.797	1.659	1.262	1.064
	1.691	1.973	1.422	1.143
	2.025	2.054	1.093	1.474
Leaves	4.230	4.772	4.090	4.416
	4.347	4.641	4.256	3.606
	4.332	4.913	4.803	4.346
	5.193	4.906	4.267	4.250
Husks			4.078	4.320
			3.820	2.500
			3.867	2.811
			3.041	2.556
Seeds			5.603	
			5.491	4.727
			5.673	5.146
			5.623	

Appendix 6. N content (%) in root dry matter of crops at Breton plots in 1987

Depth (cm)	1st	2nd	3rd	4th sampling
		Barley		
0-10	1.170	1.118	1.249	1.232
	1.206	1.038	1.206	1.302
	0.996	1.538	0.871	1.354
	1.103	1.010	0.937	1.135
10-20	1.300	1.382	1.543	1.460
	1.591	0.939	1.474	1.358
	1.292	1.294	1.817	1.419
	1.196	1.367	1.583	1.580
20-27	1.443	1.485	1.725	1.598
	1.520	1.348	1.288	1.436
	1.436	1.266	1.665	1.709
	1.236	1.430	1.560	1.376
		Canola		
0-10	1.665	0.840	1.138	1.372
	1.462	0.985	0.979	1.079
	1.409	1.279	0.991	1.148
	1.454	0.880	1.105	1.243
10-20	1.586	1.390	1.547	1.552
	1.606	1.368	1.556	1.333
	1.363	1.455	1.530	1.370
	1.278	1.384	1.533	1.193
20-27	1.732	1.558	1.846	1.505
	2.557	1.520	1.512	1.544
	1.653	1.685	1.472	1.467
	1.304	3.400	1.491	1.440
		Faba bean		
0-10	2.221	2.101	1.936	2.214
	2.205	2.288	1.675	1.696
	3.087	2.049	1.703	2.077
	2.475	2.230	1.760	1.863
10-20	2.057	1.572	2.016	1.549
	1.981	2.151	2.272	1.625
	2.337	1.908	2.491	1.863
	2.498	2.136	1.801	2.035
20-27	1.927	2.019	1.625	2.072
	1.940	2.049	1.850	1.830
	2.455	1.915	2.205	2.002
	1.954	2.544	1.889	2.065

Appendix 7. N content (%) in organic residues at Breton plots

Depth (cm)	1st	2nd	3rd	4th sampling
		Control		
0-10	1.053	1.184	0.947	1.091
	0.938	0.883	0.892	0.983
	1.084	0.980	1.118	0.999
	1.163	1.229	0.976	1.110
10-20	0.734	0.826	0.869	0.858
	0.734	0.770	0.609	0.728
	0.913	0.714	0.751	0.911
	0.654	0.662	0.916	0.830
20-27	0.618	0.948	0.816	0.981
	0.841	0.871	0.753	0.868
	0.542	1.058	0.989	1.102
	0.874	0.690	0.984	0.998
		Barley		
0-10	1.002	0.937	1.076	0.855
	1.115	1.094	1.108	1.029
	1.074	1.022	0.973	1.026
	1.028	1.043	0.966	1.006
10-20	0.647	0.596	0.667	0.676
	0.933	0.864	0.852	0.800
	0.942	0.907	0.857	1.007
	0.791	0.667	0.581	0.902
20-27	0.819	0.635	0.675	0.718
	0.828	0.696	0.916	0.898
	0.487	0.687	0.733	1.032
	0.689	0.728	0.887	0.736
		Canola		
0-10	0.972	0.920	1.178	0.984
	1.169	1.137	1.031	1.094
	1.125	0.861	1.193	1.117
	1.208	0.926	1.135	0.857
10-20	0.599	0.688	0.664	0.776
	0.929	0.718	0.753	0.695
	0.756	0.805	0.848	1.034
	0.810	0.692	0.694	0.864
20-27	0.568	0.608	0.618	0.699
	0.570	0.844	0.659	0.647
	0.928	0.882	0.842	0.865
	0.741	0.687	0.690	0.651

(continued)

Faba bean

0-10	0.985	0.983	1.085	1.144
	1.142	1.291	1.255	1.000
	1.521	1.047	1.136	1.086
	1.368	0.938	1.882	1.374
10-20	1.149	0.757	0.886	0.691
	0.875	0.849	1.021	0.843
	0.904	0.712	0.767	0.804
	0.751	0.794	0.858	1.025
20-27	0.943	0.623	0.976	0.813
	1.273	0.697	0.969	0.955
	1.092	0.629	0.867	0.787
	0.477	0.608	0.715	1.085

Appendix 8. C content (%) in crops and soil at Breton

Component	Faba bean	Canola	Barley
		Tops	
Live-leaves	42.41	43.21	45.54
Dead-leaves			43.18
Stems	45.60	45.78	45.98
Sheaths			45.06
Head			44.44
Husks	46.52	44.43	40.59
Seeds	47.10	45.68	45.89
		Roots*	
0-10cm	33.82	38.58	42.42
	41.96	42.23	41.77
10-20cm	33.73	33.76	30.19
	34.69	31.27	43.87
20-27cm	35.60	37.96	41.26
	34.40	40.55	43.10
		Residue from last cropping	
0-10cm		36.08	
10-20cm		39.62	
20-27cm		30.75	

	Soils			
	Faba bean	Canola	Barley	Summer fallow
0-10cm	2.52	2.20	2.54	2.18
	2.65	2.25	2.66	2.32
10-20cm	1.96	2.06	1.43	2.18
	1.65	1.36		
20-27cm	1.97	1.05	0.88	0.80
	1.90	0.84	0.77	0.61
0-10cm	2.45	2.57	2.57	2.64
	2.64	2.58	2.66	2.27
10-20cm	1.91	1.79	1.57	1.81
	.97	1.05	0.88	0.80
20-27	0.91	0.82	0.65	0.90

* Data were analyses oo samples from the 1st and last sampling

Appendix 9. N content (%) in soil at Breton plots in 1987

Depth (cm)	1st	2nd	3rd	4th sampling
		Control		
0-10	0.184	0.176	0.189	0.180
	0.205	0.205	0.203	0.206
	0.210	0.215	0.215	0.207
	0.169	0.169	0.169	0.178
10-20	0.166	0.135	0.150	0.150
	0.194	0.119	0.150	0.152
	0.201	0.155	0.142	0.130
	0.128	0.120	0.121	0.099
20-27	0.056	0.072	0.080	0.054
	0.066	0.058	0.066	0.076
	0.064	0.077	0.072	0.059
	0.061	0.074	0.064	0.054
		Barley		
0-10	0.201	0.188	0.203	0.199
	0.198	0.188	0.203	0.196
	0.175	0.186	0.170	0.204
	0.206	0.200	0.201	0.207
10-20	0.132	0.137	0.181	0.130
	0.147	0.162	0.171	0.126
	0.129	0.104	0.194	0.152
	0.184	0.159	0.109	0.156
20-27	0.058	0.065	0.100	0.056
	0.059	0.062	0.062	0.058
	0.052	0.075	0.062	0.058
	0.059	0.075	0.066	0.073
		Canola		
0-10	0.182	0.162	0.184	0.184
	0.202	0.188	0.205	0.206
	0.195	0.181	0.204	0.201
	0.19	0.193	0.200	0.186
10-20	0.145	0.130	0.130	0.133
	0.128	0.133	0.102	0.137
	0.147	0.135	0.132	0.154
	0.142	0.131	0.147	0.098
20-27	0.065	0.072	0.066	0.057
	0.061	0.067	0.066	0.055
	0.055	0.076	0.061	0.065
	0.059	0.072	0.074	0.049

(continued)

Faba bean

0-10	0.208	0.194	0.187	0.207
	0.198	0.178	0.192	0.199
	0.205	0.207	0.218	0.212
	0.202	0.178	0.195	0.187
10-20	0.158	0.146	0.121	0.150
	0.165	0.110	0.136	0.120
	0.143	0.148	0.141	0.145
	0.135	0.141	0.178	0.109
20-27	0.075	0.071	0.064	0.057
	0.064	0.062	0.066	0.049
	0.062	0.071	0.071	0.061
	0.064	0.072	0.095	0.053

Appendix 10. N-15 abundance (%) of above ground components of crops

Components	1st	2nd	3rd	4th sampling
		Barley		
Stems	2.08005	2.92468	2.76264	4.17015
	3.41541	3.49919	2.92215	2.88206
	1.79437	3.60226	2.80205	1.49856
	3.07710	3.08990	3.14865	3.36793
Live leaves	2.06656	2.62350		
	3.16584	3.05831		
	1.63693	3.43550		
	2.22278	3.45911		
Dead leaves	0.76341	1.28406	2.62540	2.63505
	0.75646	1.56150	1.90034	4.01096
	0.74257	1.29618	2.41835	3.18150
	0.65995	1.83123	2.31022	3.70970
Sheaths	2.18024	2.87612	2.95164	3.89889
	3.39245	3.54243	3.08113	3.65719
	1.95740	3.50154	3.13812	3.18150
	2.99102	3.28907	3.30201	3.70970
Heads		3.11328		
		3.59065		
		3.98923		
		3.77061		
Seeds			3.88830	4.48406
			4.37224	4.21602
			4.75253	3.65628
			4.25196	3.92577
Husks			3.24490	4.40724
			3.03425	3.62182
			2.78934	2.51618
			3.36807	3.71520
		Canola		
Stems	2.49447	3.22105	2.07609	3.89484
	2.68459	2.94435	3.67949	4.18344
	2.35858	3.04049	3.51695	4.17344
	2.02076	3.29051	3.79252	5.56033
Leaves	2.34480	3.48446	2.30856	
	2.73817	3.39396	4.21882	
	2.39387	3.54173	3.96942	
	2.06226	2.92468	4.13871	

Shells			2.58563	4.63363
			3.68729	4.11295
			4.03843	4.60789
			4.29686	4.97845
Seeds			2.47910	4.76682
			3.13620	4.40558
			4.20172	4.77353
			3.74708	5.08016
		Faba bean		
Stems	0.85768	1.48078	1.02294	1.05492
	0.91098	1.24302	0.94624	1.06956
	0.64215	0.87932	1.16641	0.81681
	0.54872	1.14167	0.72089	1.56155
Leaves	0.78146	1.48540	1.04934	1.04820
	0.83815	1.17584	0.95520	1.04690
	0.59803	0.84819	1.16309	1.04820
	0.51517	1.06594	0.70750	1.04820
Shells			0.81387	1.26566
			0.84578	1.04222
			1.02186	0.73258
			0.67430	1.14822
Seeds			0.74550	
			0.80996	1.02365
			1.00347	0.75855
			0.59495	

Appendix 11. N-15 abundance (%) in root dry matter of crops at Breton plots

Depth (cm)	1st	2nd	3rd	4th sampling
		Barley		
0-10	1.23587	1.26094	2.06593	1.65922
	1.32679	1.48706	1.25271	1.90487
	0.90485	1.25911	1.58573	0.90936
	1.14080	2.06944	1.56293	2.31019
10-20	0.64560	0.86043	1.06363	1.87779
	0.66143	1.04144	0.65795	0.99084
	0.51776	0.97389	0.69114	0.64089
	0.59827	1.36947	0.70368	0.75292
20-27	0.60379	0.82886	0.77764	0.67734
	0.65618	0.86291	0.72429	0.88669
	0.52762	0.58288	0.66751	0.50700
	0.50500	0.63577	0.72315	0.53184
		Canola		
0-10	1.41420	2.49827	1.76845	1.88775
	1.50314	1.71423	2.47570	2.41422
	1.21464	1.70233	1.98883	2.67407
	1.20350	2.21921	2.22815	2.55225
10-20	0.74760	0.83194	0.80822	0.78799
	0.98658	1.06815	0.76760	0.93461
	0.83286	0.88450	0.91888	0.72971
	0.61745	1.02496	0.81762	1.28173
20-27	0.46854	0.77109	0.93346	0.71086
	0.75371	0.65522	0.90363	0.80377
	0.69772	0.82354	0.81811	0.75829
	0.50112	0.54561	1.05278	1.47507
		Faba bean		
0-10	1.08738	1.38031	1.14923	1.49193
	0.81536	1.05243	1.06875	1.04244
	0.62116	1.15958	1.19250	0.95078
	0.67991	1.25128	0.74063	1.34577
10-20	0.62633	0.93757	0.69659	1.06827
	0.51521	0.70385	0.71348	1.02046
	0.49133	0.86293	0.92153	0.79950
	0.49962	0.90970	0.73980	0.85881
20-27	0.52751	0.71138	0.62727	0.67725
	0.41859	0.63856	0.65568	0.82149
	0.51123	0.65069	0.80695	0.59520
	0.60783	0.80780	0.73722	0.66384

Appendix 12. N-15 abundance (%) in organic residues at Breton plots

Depth (cm)	1st	2nd	3rd	4th sampling
		Control		
0-10	0.42765	0.56898	0.53736	0.67268
	0.44400	0.50223	0.54001	0.75544
	0.43701	0.55471	0.61513	0.71208
	0.43943	0.51925	0.60514	0.66755
10-20	0.37067	0.84287	0.38509	0.40122
	0.37320	0.38302	0.37721	0.39011
	0.37137	0.38156	0.39883	0.43579
	0.37805	0.37995	0.38956	0.41105
20-27	0.38032	0.43015	0.39650	0.41296
	0.39395	0.39460	0.42364	0.41234
	0.37870	0.40404	0.45080	0.54296
	0.39157	0.39559	0.44135	0.48603
		Barley		
0-10	0.45557	0.52969	0.69236	0.63178
	0.47448	0.56857	0.59204	0.73925
	0.44227	0.62054	0.70718	0.94773
	0.46087	0.55172	0.60587	0.81349
10-20	0.37598	0.38418	0.39195	0.41291
	0.37762	0.38180	0.42205	0.43976
	0.37439	0.38786	0.38418	0.41744
	0.37968	0.40627	0.39283	0.41987
20-27	0.37751	0.41283	0.38253	0.47750
	0.38670	0.40344	0.41642	0.46298
	0.38297	0.43139	0.46259	0.42187
	0.38065	0.39595	0.41511	0.47215
		Canola		
0-10	0.46653	0.62083	0.66290	0.76283
	0.47684	0.50074	0.63821	0.71592
	0.48613	0.68313	0.66355	0.96078
	0.47891	0.58639	0.66507	0.86161
10-20	0.38161	0.38038	0.38698	0.40643
	0.39599	0.37609	0.39826	0.41369
	0.38078	0.38685	0.42676	0.42873
	0.37960	0.37638	0.39525	0.44857
20-27	0.33815	0.38666	0.48571	0.46443
	0.37816	0.38425	0.44672	0.48145
	0.38949	0.42248	0.46507	0.43251
	0.38815	0.37448	0.42762	0.52566

Faba bean

0-10	0.47553	0.62324	0.75280	0.87541
	0.49344	0.55466	0.62113	0.80956
	0.49890	0.59630	0.74242	0.78297
	0.54828	0.72186	0.77608	0.76934
10-20	0.39961	0.42306	0.42881	0.48081
	0.37970	0.40650	0.45101	0.47259
	0.37712	0.40200	0.44140	0.43652
	0.38894	0.41851	0.44975	0.45076
20-27	0.39243	0.40101	0.52471	0.47810
	0.37775	0.38712	0.53355	0.66944
	0.44005	0.40848	0.46139	0.45047
	0.45447	0.42581	0.61915	0.49843

Appendix 13. N-15 abundance (%) in soil at Breton plots

Depth (cm)	1st	2nd	3rd	4th sampling
		Control		
0-10	0.40620	0.41628	0.44137	0.46829
	0.39668	0.41574	0.41588	0.45766
	0.39575	0.41657	0.42641	0.43837
	0.41444	0.43171	0.40304	0.43131
10-20	0.37602	0.39928	0.37459	0.41676
	0.37599	0.39522	0.38814	0.37877
	0.37730	0.38768	0.37969	0.37824
	0.37695	0.39977	0.37958	0.39504
20-27	0.37752	0.38589	0.37597	0.41136
	0.37718	0.39122	0.40640	0.38718
	0.37749	0.38465	0.38132	0.38490
	0.37744	0.38390	0.38517	0.41833
		Barley		
0-10	0.39529	0.40723	0.40532	0.44729
	0.39488	0.40193	0.44115	0.42158
	0.39324	0.39519	0.42547	0.42526
	0.38988	0.42100	0.43584	0.4550
10-20	0.37585	0.37620	0.37337	0.37398
	0.37616	0.37753	0.37313	0.37479
	0.37648	0.37779	0.37377	0.37376
	0.37617	0.37743	0.37400	0.37362
20-27	0.37974	0.37238	0.37404	0.37067
	0.37817	0.37241	0.37392	0.37286
	0.37827	0.37460	0.37391	0.37216
	0.37716	0.37375	0.37405	0.37323
		Canola		
0-10	0.38251	0.40959	0.41438	0.45780
	0.37956	0.40981	0.42292	0.43164
	0.40330	0.40816	0.44655	0.43421
	0.38857	0.40288	0.41998	0.44237
10-20	0.37638	0.37609	0.37479	0.37470
	0.37776	0.37667	0.37557	0.37480
	0.37661	0.37677	0.37667	0.37425
	0.37633	0.37734	0.38021	0.37545
20-27	0.37701	0.36807	0.37516	0.37330
	0.37709	0.36877	0.37387	0.37536
	0.37726	0.37060	0.37408	0.37220
	0.37675	0.37064	0.37460	0.37036

(continued)

Faba bean

0-10	0.39648	0.41750	0.45204	0.45079
	0.40717	0.41881	0.44898	0.45540
	0.39980	0.42884	0.40360	0.45584
	0.38768	0.41233	0.43569	0.42823
10-20	0.37636	0.37754	0.37555	0.37564
	0.37617	0.37973	0.37797	0.37744
	0.37634	0.37655	0.37472	0.37584
	0.37813	0.37671	0.37741	0.37559
20-27	0.37734	0.36781	0.37358	0.37161
	0.37658	0.36610	0.37363	0.37651
	0.37682	0.36629	0.37381	0.37441
	0.37633	0.38096	0.37412	0.37306

Appendix 14. Analysis data for soils cropped at Breton*

Crop	Date	Depth(cm)	Mineral N	N-15 abundance
			NH_4^+ (µg N/g soil)	
Faba bean	July 8	0-10	0.27	0.44263
			0.49	0.44651
Canola			0.32	0.44752
			0.61	0.43616
Barley			0.22	0.42193
			0.43	0.41855
Summer fallow			0.33	0.43746
			0.36	0.43750
			NO_3^- (µg N/g soil)	
Faba bean	July 8	0-10	8.56	1.15813
			8.19	1.14004
Canola			3.56	1.06896
			4.40	1.10518
Barley			3.93	0.73002
			3.39	0.72567
Summer fallow			13.17	1.96601
			13.76	1.94494
			NH_4^+ (µg N/g soil)	
Faba bean	July 24	0-10	0.28	0.44639
			0.51	0.44040
Canola			0.23	0.42965
			0.50	0.42816
Barley			0.12	0.43566
			0.17	0.43567
Summer fallow			0.12	0.43884
			0.09	0.44005
			NO_3^- (µg N/g soil)	
Faba bean	July 24	0-10	5.08	1.44324
			5.83	1.42962
Canola			3.12	0.75339
			2.75	0.78924
Barley			4.25	0.91845
			4.26	0.92094
Summer fallow			5.43	1.86825
			7.81	2.00192

			NH_4^+ (µg N/g soil)	
Faba bean	Aug 19	0-10	0.39	0.67677
			0.49	0.59003
Canola			0.46	0.49720
			0.31	0.47571
Barley			0.30	0.51997
			0.53	0.50446
Summer fallow			0.52	0.52746
			0.36	0.50992
			NO_3^- (µg N/g soil)	
Faba bean	Aug 19	0-10	5.45	1.21110
			6.15	1.22619
Canola			5.68	0.82372
			5.44	0.81132
Barley			5.66	0.97429
			5.90	0.97875
Summer fallow			6.31	1.11462
			5.60	2.11616
			NH_4^+ (µg N/g soil)	
Faba bean	Sept 1	0-10	0.36	0.38180
			0.39	0.38930
Canola			0.52	0.38729
			0.51	0.37790
Barley			0.39	0.38164
			0.51	0.37297
Summer fallow			0.09	0.38321
			0.24	0.37898
			NO_3^- (µg N/g soil)	
Faba bean	Sept 1	0-10	8.33	0.50247
			7.44	0.50290
Canola			7.86	0.49723
			6.79	0.49834
Barley			8.75	0.46109
			9.95	0.45875
Summer fallow			6.77	2.19360
			6.44	2.22258
			NH_4^+ (µg N/g soil)	
Faba bean	Sept 1	10-20	0.22	0.66641
			0.12	0.65878
Canola			0.08	0.52422
			0.09	0.52550
Barley			0.04	0.51280
			0.13	0.49260
Summer fallow			0.09	0.48230
			0.08	0.47606

			NO_3^- (µg N/g soil)	
Faba bean	Sept 1	10-20	0.56	1.90345
			0.85	1.87027
Canola			0.21	1.47057
			0.65	1.40574
Barley			0.17	1.68858
			0.27	1.67573
Summer fallow			3.35	2.49436
			3.59	2.44319

* These actually are at time zreo before fumigation and incubation.

Appendix 15. Analysis data for soils fumigated and incubated at Breton

Crop	Date	Depth(cm)	Mineral N	N-15 abundance
		NH_4^+ (µg N/g soil)		
Faba bean	July 8	0-10	21.16	0.60384
			22.02	0.58912
Canola			23.79	0.65428
			23.29	0.65105
Barley			27.61	0.54587
			22.41	0.55487
Summer fallow			22.78	0.55933
			21.71	0.57491
		NO_3^- (µg N/g soil)		
Faba bean	July 8	0-10	8.71	1.15441
			9.42	1.14816
Canola			5.09	1.10161
			4.65	1.07577
Barley			4.98	0.71148
			3.36	0.70359
Summer fallow			13.07	1.90399
			17.15	1.99874
		NH_4^+ (µg N/g soil)		
Faba bean	July 24	0-10	26.65	0.75706
			16.95	0.74808
Canola			19.27	0.64533
			22.11	0.64576
Barley			20.64	0.72273
			20.87	0.72299
Summer fallow			20.07	0.62093
			20.00	0.60160
		NO_3^- (µg N/g soil)		
Faba bean	July 24	0-10	5.37	1.25427
			5.15	1.37280
Canola			3.60	0.79186
			4.18	0.80230
Barley			5.21	0.92766
			6.10	0.93698
Summer fallow			13.66	2.11894
			14.11	2.08651

		NH_4^+ (µg N/g soil)		
Faba bean	Aug 19	0-10	21.21	0.90705
			22.98	0.90650
Canola			23.19	0.70557
			23.01	0.68673
Barley			25.60	0.89180
			25.12	0.86155
Summer fallow			21.88	0.79817
			21.69	0.77205
		NO_3^- (µg N/g soil)		
Faba bean	Aug 19	0-10	3.78	1.10278
			4.82	1.07972
Canola			4.54	0.78431
			5.93	0.81641
Barley			4.43	0.98150
			4.32	0.96002
Summer fallow			6.66	1.10961
			5.66	1.08995
		NH_4^+ (µg N/g soil)		
Faba bean	Sept 1	0-10	26.28	1.26627
			24.77	1.25297
Canola			26.01	1.09323
			26.19	1.08113
Barley			24.22	0.97332
			25.57	1.02725
Summer fallow			22.22	0.87644
			21.41	0.90392
		NO_3^- (µg N/g soil)		
Faba bean	Sept 1	0-10	6.18	1.65134
			6.81	1.69434
Canola			5.03	1.33797
			5.65	1.34028
Barley			7.41	1.52074
			8.02	1.54830
Summer fallow			8.84	2.32608
			8.87	2.29314
		NH_4^+ (µg N/g soil)		
Faba bean	Sept 1	10-20	16.41	0.47657
			13.94	0.55361
Canola			14.18	0.44709
			13.68	0.43070
Barley			12.41	0.40812
			11.98	0.40301
Summer fallow			12.82	0.43592
			12.75	0.42252

			NO_3^- (µg N/g soil)	
Faba bean	Sept 1	10-20	2.61	0.50606
			2.61	0.49853
Canola			2.13	0.48600
			2.86	0.49339
Barley			2.06	0.47960
			1.86	0.46954
Summer fallow			6.56	2.28673
			6.06	2.22963

Appendix 16. Analysis data for soils nonfumigated and incubated at Breton

Crop	Date	Depth(cm)	Mineral N	N-15 abundance
			NH_4^+ (µg N/g soil)	
Faba bean	July 8	0-10	1.22	0.44744
			0.85	0.49398
Canola			0.96	0.43736
			1.06	0.44873
Barley			0.39	0.42742
			0.14	0.42001
Summer fallow			0.39	0.46000
			0.79	0.46187
			NO_3^- (µg N/g soil)	
Faba bean	July 8	0-10	12.53	1.10540
			11.71	1.12400
Canola			5.65	1.00329
			7.00	1.02413
Barley			6.98	0.70290
			6.00	0.69075
Summer fallow			22.34	1.94064
			17.27	1.93198
			NH_4^+ (µg N/g soil)	
Faba bean	July 24	0-10	0.63	0.55848
			0.60	0.54865
Canola			0.44	0.46499
			0.56	0.46302
Barley			0.49	0.45527
			0.44	0.46430
Summer fallow			0.42	0.43321
			0.56	0.44645
			NO_3^- (µg N/g soil)	
Faba bean	July 24	0-10	7.79	1.41389
			8.83	0.86713
Canola			6.63	0.80135
			7.20	0.80230
Barley			8.86	0.93977
			7.34	0.95573
Summer fallow			15.62	2.07660
			13.95	1.97286

		NH_4^+ (µg N/g soil)		
Faba bean	Aug 19	0-10	1.00	0.57734
			0.87	0.56264
Canola			0.71	0.45753
			0.69	0.45612
Barley			0.52	0.52211
			0.96	0.53548
Summer fallow			0.75	0.50984
			0.59	0.50469
		NO_3^- (µg N/g soil)		
Faba bean	Aug 19	0-10	6.47	1.15018
			6.59	1.14142
Canola			5.89	0.80915
			6.98	0.80621
Barley			4.25	0.95292
			4.68	0.98021
Summer fallow			7.40	1.07413
			5.52	1.01495
		NH_4^+ (µg N/g soil)		
Faba bean	Sept 1	0-10	1.00	0.67490
			0.94	0.74285
Canola			0.80	0.69502
			0.68	0.66961
Barley			0.90	0.65130
			1.51	1.52600
Summer fallow			0.76	0.51456
			0.35	0.54610
		NO_3^- (µg N/g soil)		
Faba bean	Sept 1	0-10	12.25	1.75890
			12.54	1.77749
Canola			7.39	1.35121
			9.02	1.39210
Barley			8.50	1.49632
			10.47	1.53970
Summer fallow			8.23	2.17426
			7.05	2.14872
		NH_4^+ (µg N/g soil)		
Faba bean	Sept 1	10-20	1.50	0.42309
			0.90	0.41723
Canola			0.91	0.39135
			0.53	0.38561
Barley			0.81	0.38503
			1.14	0.38931
Summer fallow			0.67	0.40007
			0.69	0.39798

			NO_3^- (µg N/g soil)	
Faba bean	Sept 1	10-20	3.44	0.52043
			3.06	0.52119
Canola			2.64	0.49362
			2.70	0.49731
Barley			2.50	0.46919
			2.77	0.47153
Summer fallow			5.88	2.07338
			6.28	2.11955

Appendix 17. N-15 incorporated into the flush N (ng 15N g^{-1} soil)

	July 8	July 24	August 19	September 1
Faba bean				
Method 1	29.11	46.05	97.72	135.91
Method 2	48.63	83.27	117.51	224.76
Method 3	49.66	84.42	119.42	228.07
Canola				
Method 1	60.83	41.80	70.00	155.09
Method 2	66.64	57.29	75.56	185.80
Method 3	67.41	57.78	76.20	188.14
Barley				
Method 1	38.54	56.56	127.87	129.85
Method 2	45.76	73.57	128.25	149.13
Method 3	45.92	74.00	129.47	158.01
Summer fallow				
Method 1	54.10	44.15	91.44	148.20
Method 2	44.07	48.72	90.30	113.35
Method 3	44.63	49.08	91.25	114.23

Method 1, 2 and 3 were calculated as follows:

Method 1: $F(NH_4^+ + NO_3^-)_{10} - F(NH_4^+ + NO_3^-)_0$

Method 2: $F(NH_4^+)_{10} - F(NH_4^+)_0$

Method 3: $F(NH_4^+)_{10}$

Appendix 18. Moisture contents (%) in soils at the four samplings

Treatment	July 8	July 24	August 19	September 1
		0-10cm		
Faba bean	15.5	13.8	21.5	18.5
	15.3	14.1	21.5	18.4
Canola	15.2	16.8	21.5	20.8
	15.2	16.9	21.6	20.6
Barley	17.1	16.9	23.0	23.5
	17.2	17.2	22.9	23.6
Control	18.5	20.1	22.8	20.3
	19.0	19.6	22.7	20.5

Appendix 19. Algal growth in the cylinders on last sampling

Treatment	1	2	3	4
Faba bean	+	+	+	+
Canola	+	+	++	+
Barley	++	++	+	+++
Summer fallow	++	+++	++	++

Appendix 20. Dry weight for crops from experiment conducted in China

Plot	Depth(cm)	Corn	Soybean
		Crop tops (g/pot)	
1-1		200	3.63
1-2		210	3.18
2-1		250	3.65
2-2		150	14.7
3-1		200	
3-2		300	
		Crop roots (g/pot)	
1-1	0-10	5.89	1.04
	10-20	0.76	0.00
	20-30	0.35	0.00
1-2	0-10	25.4	1.16
	10-20	7.17	0.00
	20-30	0.32	0.00
2-1	0-10	15.66	1.17
	10-20	0.22	2.03
	20-30	0.17	0.00
2-2	0-10	11.26	3.03
	10-20	2.05	0.59
	20-30	0.27	0.00
3-1	0-10	3.64	
	10-20	0.47	
	20-30	0.00	
3-2	0-10	11.14	
	10-20	1.05	
	20-30	0.53	

Appendix 21. Analysis data for crops from experiment conducted in China*

Crop	Depth(cm)	Total N(%)	N-15 abundance
		SOIL	
Corn	0-10	0.101	0.39791
	0-10	0.170	0.39133
	0-10	0.132	0.39618
	0-10	0.145	0.39303
	0-10	0.168	0.38696
	0-10	0.142	0.38913
Corn	10-20	0.063	0.38174
	10-20	0.125	0.37452
	10-20	0.149	0.37869
	10-20	0.135	0.37760
	10-20	0.167	0.37443
	10-20	0.109	0.37974
Corn	20-30		
	20-30		
	20-30	0.130	0.37571
	20-30	0.090	0.37852
	20-30	0.080	0.37672
	20-30	0.040	0.37822
Soybean	0-10	0.141	0.39709
	0-10	0.134	0.39487
	0-10	0.100	0.37747
	0-10	0.152	0.39609
	10-20	0.074	0.38026
	10-20	0.129	0.37618
	10-20	0.140	0.39416
	10-20	0.081	0.37966
	20-30	0.040	0.37797
	20-30	0.070	0.37750
	20-30	0.090	0.37755
	20-30	0.090	0.38034
		Crop tops	
Corn		0.843	0.45900
		0.556	0.66698
		0.880	0.64217
		0.942	0.61671
		0.981	0.55945
		1.028	0.55425
Soybean		1.534	0.67146
		1.198	0.59485
		1.361	0.54098
		2.953	0.44994

(continued)

		Crop roots	
Corn	0-10	0.438	0.79780
	0-10	0.356	0.65539
	0-10	0.444	0.72118
	0-10	0.367	0.71798
	0-10	0.685	0.67358
	0-10	0.645	0.62882
Corn	10-20	0.560	0.88772
	10-20	0.404	0.53988
	10-20	0.460	0.68233
	10-20	0.562	0.65921
	10-20	1.509	0.47862
	10-20	0.657	0.78677
Corn	20-30	0.481	0.95848
	20-30	0.526	0.64650
	20-30	0.526	1.13244
	20-30	0.777	0.65917
	20-30	0.589	0.63725
Soybean	0-10	0.141	0.39709
	0-10	0.134	0.39487
	0-10	0.100	0.37747
	0-10	0.152	0.39609
	10-20	0.074	0.38026
	10-20	0.129	0.37618
	10-20	0.140	0.39416
	10-20	0.081	0.37966

* This experiment was conducted to quantify dinitrogen fixation in soybean with corn as a nonfixing reference crop.

Appendix 22. Total N content (%) data of laboratory urea diffusion

Depth(cm)	Days after application			
	20	40	60	60
		Applied 2cm		
0-3	0.298	0.277	0.320	0.297
3-6	0.275	0.241	0.253	0.247
6-9	0.221	0.232	0.220	0.221
9-12	0.204	0.228	0.203	0.203
12-15	0.203	0.215	0.201	0.200
		Applied 4cm		
0-3	0.234	0.257	0.271	0.274
3-6	0.286	0.272	0.259	0.250
6-9	0.273	0.248	0.242	0.241
9-12	0.211	0.224	0.215	0.215
12-15	0.201	0.231	0.203	0.208
		Applied 8cm		
0-3	0.189	0.197	0.213	0.233
3-6	0.208	0.220	0.220	0.220
6-9	0.254	0.267	0.244	0.218
9-12	0.278	0.262	0.248	0.215
12-15	0.248	0.239	0.218	0.226

Appendix 23. N-15 abundance data of laboratory urea diffusion

Depth(cm)	Days after application			
	20	40	60	60
		Applied 2cm		
0-3	0.62921	0.62463	0.67851	0.65695
3-6	0.62604	0.59360	0.56544	0.57021
6-9	0.46508	0.50738	0.46919	0.49257
9-12	0.39553	0.41083	0.40619	0.42677
12-15	0.37576	0.38797	0.38504	0.40918
		Applied 4cm		
0-3	0.49593	0.56706	0.61011	0.61020
3-6	0.65016	0.60332	0.57647	0.57674
6-9	0.60493	0.55196	0.54123	0.54194
9-12	0.41192	0.47701	0.44829	0.46053
12-15	0.37613	0.46250	0.39537	0.43389
		Applied 8cm		
0-3	0.37916	0.39936	0.43684	0.50596
3-6	0.41021	0.46726	0.49165	0.51276

6-9	0.56528	0.61562	0.60333	0.53265
9-12	0.64054	0.60060	0.59671	0.55619
12-15	0.54597	0.48167	0.50600	0.54751

Appendix 24. ANOVA table of crop dry matter in tops

Source	DF	SS	MS
Crop	2	1255436.45	627718.23
Date	3	1455842.10	485280.70
Crop x Date	6	791965.16	131994.19
Error	36	936848.38	26023.57

Appendix 25. ANOVA table of N contents in crop tops

Source	DF	SS	MS
Crop	2	27.92	13.96
Date	3	6.76	2.25
Crop x Date	6	1.92	0.32
Error	36	1.23	0.03

Appendix 26. ANOVA table of N in crop tops

Source	DF	SS	MS
Crop	2	1802.08	901.04
Date	3	407.27	135.76
Crop x Date	6	509.36	84.89
Error	36	501.32	13.93

Appendix 27. ANOVA table of dry matter of crop roots

Source	DF	SS	MS
Crop	2	74615.65	37307.83
Date	3	17316.05	5772.02
Depth	2	233294.65	116647.32
Crop x Date	6	14859.53	2476.59
Crop x Depth	4	92305.11	23076.28
Date x Depth	6	22804.81	3800.80
Crop x Date x Depth	12	18942.94	1578.58
Error	108	91004.90	842.64

Appendix 28. ANOVA table of N content of crop roots

Source	DF	SS	MS
Crop	2	13.74	6.87
Date	3	0.51	0.17
Depth	2	1.63	0.81
Crop x Date	6	1.03	0.17
Crop x Depth	4	1.69	0.42
Date x Depth	6	1.29	0.21
Crop x Date x Depth	12	0.86	0.07
Error	108	6.89	0.06

Appendix 29. ANOVA table of N accumulated in crop roots

Source	DF	SS	MS
Crop	2	39.93	19.97
Date	3	3.68	1.23
Depth	2	64.93	32.46
Crop x Date	6	3.51	0.59
Crop x Depth	4	50.47	12.62
Date x Depth	6	4.32	0.72
Crop x Date x Depth	12	4.10	0.36
Error	108	22.48	0.21

Appendix 30. ANOVA table of N-15 abundance in crop tops

Source	DF	SS	MS
Crop	2	10.38	3.46
Date	3	58.32	29.16
Crop x Date	6	4.12	0.69
Error	36	5.77	0.16

Appendix 31. ANOVA table of N-15 abundance in crop roots

Source	DF	SS	MS
Crop	2	3.50	1.75
Date	3	3.24	1.08
Depth	2	17.75	8.87
Crop x Date	6	0.19	0.03
Crop x Depth	4	3.32	0.83
Date x Depth	6	0.95	0.16
Crop x Date x Depth	12	0.70	0.06
Error	108	6.01	0.06

Appendix 32. ANOVA table of respiration-C in soils

Source	DF	SS	MS
Crop	3	16090.67	5363.56
Date	3	57350.11	19116.70
Crop x Date	9	35825.92	3980.66
Error	16	30748.62	1921.79

Appendix 33. ANOVA table of soil microbial biomass-C in soils

Source	DF	SS	MS
Crop	3	640199.28	213399.76
Date	3	415357.13	138452.38
Crop x Date	9	307405.78	34156.20
Error	16	204310.47	12769.40

Appendix 34. ANOVA table of NH_4^+ by fumigation and incubation

Source	DF	SS	MS
Crop	3	15.04	5.01
Date	3	34.25	11.42
Crop x Date	9	12.36	1.37
Error	16	35.98	2.25
CFIT	1	7874.56	7874.56
CFIT x Crop	3	13.37	4.46
CFIT x Date	3	24.08	8.03
CFIT x Crop x Date	9	15.00	1.67
Error	16	34.32	2.15

Appendix 35. ANOVA table of NO_3^- by fumigation and incubation

Source	DF	SS	MS
Crop	3	322.20	107.40
Date	3	152.10	50.70
Crop x Date	9	284.58	31.62
Error	16	10.56	0.66
CFIT	1	72.61	72.61
CFIT x Crop	3	12.75	4.25
CFIT x Date	3	8.14	2.71
CFIT x Crop x Date	9	24.75	2.75
Error	16	27.00	1.69

Appendix 36. ANOVA table of flush N from soil microbial biomass

Source	DF	SS	MS
Crop	3	48.62	16.21
Date	3	66.61	22.20
Crop x Date	9	49.82	5.54
Error	16	125.51	7.97